Docket No. 69014-B/GJG/BJA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Appellants	:	Kiran K. Chada et al.
Serial No.	:	10/768,566 Group Art Unit: 1646
Filed	:	January 29, 2004 Examiner: G. Chandra
For	:	METHODS OF TREATING OBESITY AND METABOLIC DISORDERS RELATED TO EXCESS ADIPOSE TISSUE BY ADMINISTRATION OF S-FRP-5 PEPTIDE

1185 Avenue of The Americas
New York, New York 10036
December 10, 2007

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

COMMUNICATION IN RESPONSE TO NOVEMBER 8, 2007 NOTIFICATION OF NON-COMPLIANT APPEAL BRIEF

This Communication is submitted in response to the November 8, 2007 Notification of Non-Compliant Appeal Brief issued by the U.S. Patent and Trademark Office in connection with the above-identified application. A copy of the Notification is attached hereto as **Exhibit 1**. A response to the Notification is due December 8, 2007. However, since December 8, 2007 falls on a Saturday, a response filed on the next succeeding day which is not a Saturday, Sunday or Federal Holiday, i.e. Monday, December 10, 2007, is considered timely under 37 C.F.R. §1.7. Accordingly, this response is being timely filed.

The November 8, 2007 Notification indicates that Appellants' Appeal Brief filed October 15, 2007 (received by the U.S. Patent and Trademark Office on October 18, 2007) is defective for the following reasons:

(a) the "status of the claims" section must identify the status of claims filed in the application;
(b) the "evidence appendix" must include a heading before the copies of evidence being submitted;
(c) the "related proceedings appendix" is a required heading and if there is no appendix then an indication of "None" is required under this heading.

The Notice also states that an amended Appeal Brief must be filed within the relevant time limit to avoid dismissal of the Appeal.

In response, Appellants enclose herewith as **Exhibit 2** an amended Appeal Brief in compliance with 37 C.F.R. §41.37 which specifically includes amendments to address points (a)-(c) enumerated above. Specifically, the "status of the claims" section on page 7 of the amended Appeal Brief attached hereto refers to all claims filed in the application. The "evidence appendix" of the amended Appeal Brief attached hereto has been amended include a heading page before the copies of evidence being submitted (see Exhibit B). Finally, a "related proceedings appendix" has been included (see Exhibit D).

Appellants also note that the enclosed amended Appeal Brief refers to an enclosed check in the amount of $1295.00 covering (i) the fee for filing a brief in support of an appeal under 37 C.F.R. §41.20(b)(2); (ii) the fee for filing a request for

an oral hearing before the Board of Patent Appeals and Interferences in an appeal under 37 C.F.R. §41.20(b)(3); and (iii) the fee for a three-month extension of time for a small entity. Appellants further note that such a check was *previously* enclosed with the Appeal Brief as filed on October 15, 2007 and was received by the U.S. Patent and Trademark Office. Accordingly, a second check in this amount has not been included with the enclosed amended Appeal Brief.

If a telephone interview would be of assistance in advancing prosecution of the subject application, Appellants' undersigned attorney invites the Examiner to telephone him at the number provided below.

No fee is deemed necessary in connection with the filing of this Response. However, if any fee is required, authorization is hereby given to charge the amount of such fee to Deposit Account No. 03-3125

Respectfully submitted,

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I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to:
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Alexandria, VA 22313-1450.

Gary J. Gershik 12/10/07
Gary J. Gershik Date
Reg. No. 39,992

12/10/2010/14-8

GJG/BD

COPY

Notification of Non-Compliant Appeal Brief (37 CFR 41.37)	Application No. 10/768,566	Applicant(s) CHABRA ET AL.
	Examiner GYAN CHANDRA	Art Unit 1646

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

The Appeal Brief filed on *18 October 2007* is defective for failure to comply with one or more provisions of 37 CFR 41.37.

To avoid dismissal of the appeal, applicant must file anamended brief or other appropriate correction (see MPEP 1205.03) within **ONE MONTH or THIRTY DAYS** from the mailing date of this Notification, whichever is longer. **EXTENSIONS OF THIS TIME PERIOD MAY BE GRANTED UNDER 37 CFR 1.136.** NON compliant amendment Due 1: 3 4: 5

4mo
5mo
6mo

1. ☐ The brief does not contain the items required under 37 CFR 41.37(c), or the items are not under the proper heading or in the proper order.

2. ☒ The brief does not contain a statement of the status of all claims, (e.g., rejected, allowed, withdrawn, objected to, canceled), or does not identify the appealed claims (37 CFR 41.37(c)(1)(iii)).

3. ☐ At least one amendment has been filed subsequent to the final rejection, and the brief does not contain a statement of the status of each such amendment (37 CFR 41.37(c)(1)(iv)).

4. ☐ (a) The brief does not contain a concise explanation of the subject matter defined in each of the independent claims involved in the appeal, referring to the specification by page and line number and to the drawings, if any, by reference characters; and/or (b) the brief fails to: (1) identify, for each independent claim involved in the appeal and for each dependent claim argued separately, every means plus function and step plus function under 35 U.S.C. 112, sixth paragraph, and/or (2) set forth the structure, material, or acts described in the specification as corresponding to each claimed function with reference to the specification by page and line number, and to the drawings, if any, by reference characters (37 CFR 41.37(c)(1)(v)).

5. ☐ The brief does not contain a concise statement of each ground of rejection presented for review (37 CFR 41.37(c)(1)(vi))

6. ☐ The brief does not present an argument under a separate heading for each ground of rejection on appeal (37 CFR 41.37(c)(1)(vii)).

7. ☐ The brief does not contain a correct copy of the appealed claims as an appendix thereto (37 CFR 41.37(c)(1)(viii)).

8. ☒ The brief does not contain copies of the evidence submitted under 37 CFR 1.130, 1.131, or 1.132 or of any other evidence entered by the examiner **and relied upon by appellant in the appeal**, along with a statement setting forth where in the record that evidence was entered by the examiner, as an appendix thereto (37 CFR 41.37(c)(1)(ix)).

9. ☒ The brief does not contain copies of the decisions rendered by a court or the Board in the proceeding identified in the Related Appeals and Interferences section of the brief as an appendix thereto (37 CFR 41.37(c)(1)(x)).

10. ☐ Other (including any explanation in support of the above items):

2. Status of claims must identify the status of all claims filed in the application.
8. Evidence appendix must include a heading before the copies of evidence that were submitted.
9. Related proccedings appendix is a required headings and must contain copies of any decision by court or Board of Appeals, if there is no appendix then an indication of None is required under this heading.

TRACEY YOUNG
PATENT APPEAL CENTER SPECIALIST

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/768,566	01/29/2004	Kiran K. Chada	69014-B/GJG	6434

7590 11/08/2007

EXAMINER	

ART UNIT	PAPER NUMBER

Gary J. Gershik
Cooper & Dunham LLP
1185 Avenue of the Americas
New York, NY 11036

DATE MAILED: 11/08/2007

Please find below and/or attached an Office communication concerning this application or proceeding.

Docket No. 69014-B/GJG/BJA

Oral Hearing Requested

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Appellants	:	Kiran K. Chada et al.	
Serial No.	:	10/768,566	Group Art Unit: 1646
Filed	:	January 29, 2004	Examiner: G. Chandra
For	:	METHODS OF TREATING OBESITY AND METABOLIC DISORDERS RELATED TO EXCESS ADIPOSE TISSUE BY ADMINISTRATION OF S-FRP-5 PEPTIDE	

1185 Avenue of The Americas
New York, New York 10036
FILED October 15, 2007
AMENDED December 10, 2007

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

APPEAL BRIEF
AND REQUEST FOR ORAL HEARING AND PETITION FOR THREE-MONTH EXTENSION OF TIME

This appeal was taken from the Examiner's final rejection of claims 1-9 and 17 in the Final Office Action dated January 10, 2007 issued in connection with the above-identified application. The required fee for filing a brief in support of an appeal under 37 C.F.R. §41.20(b)(2) is TWO HUNDRED AND FIFTY FIVE DOLLARS ($255.00) for a small entity. Appellants also hereby request an oral hearing in connection with this appeal. The required fee for filing a request for an oral hearing before the Board of Patent Appeals and Interferences in an appeal under 37 C.F.R. §41.20(b)(3) is FIVE HUNDRED AND

FIFTEEN DOLLARS ($515.00) for a small entity. Accordingly, a check including these amounts is enclosed.

On May 10, 2007, Appellants mailed a Notice of Appeal and a Petition for One-Month Extension of Time which was received by the U.S. Patent and Trademark Office on May 14, 2007. Appellants' brief on appeal was due July 14, 2007, based on the May 14, 2007 receipt date of appellants' Notice of Appeal (37 C.F.R. § 41.37(a)(1) and M.P.E.P. §1206). Appellants hereby petition for a three-month extension of time. The fee for a three-month extension of time for a small entity is FIVE HUNDRED AND TWENTY FIVE DOLLARS ($525.00) and a check including this amount is enclosed. With a three-month extension of time, a response is now due October 14, 2007. However, since October 14, 2007 falls on a Sunday, a response filed on the next succeeding day which is not a Saturday, Sunday or Federal Holiday, i.e. Monday, October 15, 2007, is considered timely under 37 C.F.R. §1.7. Accordingly, this Appeal Brief is being timely filed.

If any additional fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 03-3125.

TABLE OF CONTENTS

1. REAL PARTY IN INTEREST

The owner of the subject application at the time of filing this brief is HM Gene, Inc., a corporation organized under the laws of Delaware and having a place of business at 675 Hoes Lane, Research Tower, Room R-603, Piscataway, NJ, 08854, the assignee of record of the above-identified patent by virtue of an assignment from Kiran K. Chada, Roland Chouinard, Hena Ashar and Md. Abu Sayed recorded on July 28, 2004 with the U.S. Patent and Trademark Office at Reel 015613 Frame 0506, a copy of which is attached hereto as **Exhibit C**.

2. RELATED APPEALS AND INTERFERENCES

None.

3. STATUS OF CLAIMS

Claims 1, 8, 9 and 17-19 as reproduced in **Claims Appendix A-1** have been entered and finally rejected solely on the ground of anticipation. Accordingly, claims 1, 8, 9 and 17-19 are pending and rejected. Claims 2-7 and 10-16 have previously been cancelled.

4. STATUS OF AMENDMENTS

On January 10, 2007, the date of issuance of the Final Office Action, claims 1-9 and 17 were pending and finally rejected in the subject application.

In their January 26, 2007 Amendment appellants presented for entry an amended claim set, including the cancellation of claims 2-7 without prejudice, the addition of new claims 18 and 19, and the amendment of claims 1 and 17. On April 16, 2007, the Examiner issued an Advisory Action indicating that the set of amended claims would be entered but did not place the subject application in condition for allowance.

5. SUMMARY OF THE CLAIMED SUBJECT MATTTER

The claimed subject matter common to independent claim 1 and dependent claims 8 and 9 is method of reducing the amount of adipose tissue in a subject comprising administering to the subject an amount of an sFRP-5 peptide effective to reduce the amount of adipose tissue, or an amount of a molecule effective to stimulate expression of the sFRP-5 peptide in the subject, wherein the sFRP-5 peptide comprises consecutive amino acids having the sequence set forth in SEQ ID NO: 1. The claimed subject matter common to independent claim 17 and dependent claims 18 and 19 is a method of reducing the level of adipose tissue formation in a subject comprising administering to the subject an amount of an sFRP-5 peptide effective to reduce the level of adipose tissue formation, or an amount of a molecule effective to stimulate expression of the sFRP-5 peptide in the subject, wherein the sFRP-5 peptide comprises consecutive amino acids having the sequence set forth in SEQ ID NO: 1.

6. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

There is one ground of rejection to be reviewed, whether Appellants' now claimed invention is anticipated under 35 U.S.C. §102(e) by Xu et al., U.S. Patent Application Publication No. 2003/0143610 A1, published July 31, 2003 ("Xu et al.") (**Evidence Appendix B.1**).

7. ARGUMENT

It is the Appellants' position that (i) Xu et al. does not teach all elements of the claimed invention; (ii) Xu et al. does not inherently disclose all elements of the claimed invention; (iii) the requirements for inherent anticipation have not been met in the rejection set forth; and (iv) Xu et al. is not an enabling disclosure for what the Examiner alleged it teaches.

Appellants note that their position iterated in the preceding paragraph applies separately to each of the rejected claims 1, 8, 9 and 17-19. Appellants further note that the arguments provided hereinbelow apply separately to each of claims 1, 8, 9 and 17-19. Thus, claims 1, 8, 9 and 17-19 do not stand together but stand separately.

7.1 THE LEGAL STANDARD FOR ANTICIPATION

7.1.1 Single prior art document must teach all elements of the claimed invention

As noted in M.P.E.P. §706.02(a) "for anticipation under 35 U.S.C. 102, the reference must teach *every aspect* of the claimed invention either explicitly or impliedly. Any feature not directly taught must be inherently present" (emphasis added).

7.1.2 Anticipation rejection based on inherency requires missing descriptive material to be necessarily present in the matter described in the prior art reference

With regard to inherent anticipation, "[t]he fact that a certain result or characteristic may occur or be present in

the prior art is not sufficient to establish the inherency of that result or characteristic. In re Rijckaert, 9 F.3d 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993)", as cited in M.P.E.P. §2112. More specifically, "[t]o establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. *Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.*' In re Robertson, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1999)" (M.P.E.P. §2112) (emphasis added).

7.1.3 The prior art document must provide an enabling disclosure for what it allegedly teaches

The M.P.E.P §2121.01 makes it clear that for a prior art reference to anticipate a claim the reference must contain an enabling disclosure for the anticipatory subject matter. "The disclosure in an assertedly anticipating reference must provide an enabling disclosure of the desired subject matter; *mere naming or description of the subject matter is insufficient*, if it cannot be produced without undue experimentation. Elan Pharm., Inc. v. Mayo Found. For Med. Educ. & Research, 346 F.3d 1051, 1054, 68 USPQ2d 1373, 1376 (Fed. Cir. 2003)" (emphasis added).

7.2 XU ET AL. DOES NOT TEACH EVERY ASPECT OF APPELLANTS' CLAIMED INVENTION

7.2.1. *Examiner's Characterization of prior art reference Xu et al.*

(Initially, Appellants note that it has been agreed that sFRP-5 peptide is the same as SARP3).

The Examiner alleged on pages 11 and 12 of the Office Action issued April 19, 2006 in connection with the above-identified application (as cited by the Examiner in the January 10, 2007 Final Office Action and in the April 16, 2007 Advisory Action) that Xu et al. teaches "administration of a polypeptide SARP of SEQ ID NO:2 which is 100% identical to the polypeptide of SEQ ID NO:1 (appendix-A) of the instant application for the treatment of metabolic disorders including obesity and diabetes comprising the SARPs polypeptides...." The Examiner further stated that Xu et al. "do not explicitly teach reduction in an amount of adipose tissue but the administration of the polypeptide of SEQ ID NO:2 or a variant would inherently achieve the same effect in a subject as instantly being claimed...."

7.2.2. *Xu et al. does not teach all the elements of the claimed invention.*

Claim 1, and claims 8 and 9 dependent therefrom, require that the subject be administered (i) an amount of an sFRP-5 peptide effective to reduce the amount of adipose tissue, or (ii) an amount of a molecule effective to stimulate expression of the sFRP-5 peptide in the subject. Xu et al. however, does not teach either of these. Instead Xu et al. discusses administering an SARP3 "modulator".

Importantly there is no teaching in Xu et al. of what the "modulator should do; no teaching whatsoever of whether the modulator should inhibit SFRP-5 or induce SFRP-5 or activate SFRP-5 production. This information is not in Xu et al.

Thus, in rejecting claims 1, 8 and 9, appellants note that the Examiner has not properly applied the law of anticipation. The same argument applies mutatis mutandis to the subject matter of claims 17-19.

Xu et al. does not teach what "modulating" SARP3 means. Although the Examiner states in the Advisory Action issued April 16, 2007 in connection with the above-identified application that Xu et al. "interpret the term 'modulate' as either to stimulate or to inhibit (see-[0041]" this is not factually correct. What paragraph [0041] of Xu et al. actually states is "..to modulate (***e.g.***, stimulate or inhibit) the activity of the SARP3 polypeptide" (emphasis added). Thus, Xu et al. provide two examples of what modulate might mean.

Clearly, the Examiner's binary interpretation of "modulate" as used by Xu et al. to mean either stimulate or inhibit is not warranted in view of the exemplification language clearly implying other encompassed embodiments. Appellants suggest, merely for the sake of argument, that other such embodiments of "modulate" might be making the activity of the SARP3 polypeptide preferentially sensitive to one compound in favor of another etc. In any event, "modulate activity of" cannot be read as synonymous with "stimulate expression of" as is required for the Examiner's anticipation rejection to be proper. The interpretation of modulate as stimulate is merely one possibility selected by the Examiner to reject appellants'

invention, and cannot be the basis of a proper anticipation rejection.

It is appellants position that Xu et al. does not explicitly teach all the elements of appellants' invention as claimed in claims 1, 8, 9, and 17-19.

7.3 MISSING DESCRIPTIVE MATERIAL NOT NECESSARILY PRESENT IN XU ET AL.

7.3.1 An anticipation rejection relying on inherency is not proper unless "the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill", M.P.E.P. §2112, as cited above. In fact inherency "may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient", M.P.E.P. §2112, as cited above. Thus for the Examiner's rejection of claim 1 (and claims 8 & 9) as based on inherency to be proper the modulatory amount of SARP3 discussed in Xu et al. *must necessarily* also be an amount effective to reduce the amount of adipose or an amount effective to stimulate expression of the sFRP-5 peptide in the subject. Similarly, for the Examiner's rejection of claim 17 (and claims 18 & 19) as based on inherency to be proper the modulatory amount of SARP3 discussed in Xu et al. must necessarily also be an amount effective to reduce the level of adipocyte formation or an amount effective to stimulate expression of the sFRP-5 peptide in the subject. Appellants note that "[i]n relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic

necessarily flows from the teachings of the applied prior art." Ex parte Levy, 17 USPQ2d 1461, 1464 (Bd. Pat. App. & Inter. 1990) (emphasis added).

Xu et al. does not teach anything which could inherently result in the claimed invention. Xu et al. does not teach either (a) administering to a subject an amount of an sFRP-5 peptide effective to reduce the amount of adipose tissue or (b) administering to a subject or an amount of a molecule effective to stimulate expression of the sFRP-5 peptide in the subject as recited in claims 1, 8 or 9. In addition, appellants maintain that Xu et al. does not explicitly or inherently teach (a) administering to a subject an amount of an sFRP-5 peptide effective to reduce the formation of adipose tissue or (b) administering to a subject or an amount of a molecule effective to stimulate expression of the sFRP-5 peptide in the subject as recited in claims 17, 18 or 19.

Appellants further note that the Examiner has not put forth any evidence demonstrating that the unstated elements in the prior art were ever present. Rather, the Examiner has relied on a statement of a guess in a patent application to conclude that the prior art must inherently disclose all of the elements of the claims. However, claims cannot be inherently anticipated if the prior art fails to disclose, and is not capable of inherently disclosing, all of the elements of the claims. *See Application of Seaborg*, 328 F.2d 996 (C.C.P.A. 1964).

The case of *Application of Glenn T. Seaborg* is illustrative of this point. In *Seaborg*, the applicant sought to patent atomic element 95. The patent office relied on a prior art patent disclosing a nuclear reactor and a theoretical formula which

predicted that the claimed element would be produced by running the reactor. *Id.* at 996-97. The Court of Customs and Patent Appeals reversed this rejection, holding that there was no positive evidence that the claimed element was inherently produced by running the nuclear reaction. *Id.* at 999.

In this case, the Examiner has relied on a guess stated in a patent application, not even an experiment. Like in *Seaborg*, the Examiner has chosen one convenient speculation from the prior art. Such hindsight selection based on speculation is not sufficient to reject a claim under the doctrine of inherent anticipation.

Appellants note with regard to this point that, in *Perricone v. Medicis Pharmaceutical Corporation*, a district court held a method claim for treating a sunburn by applying a specific compound to the affected area to be inherently anticipated by prior art disclosing compound for "topical application" to the skin. On appeal, however, the Federal Circuit reversed the finding of invalidity and determined that the prior art did not anticipate this claim because "the district court's inherency analysis goes astray because it assumes what [the prior art] neither disclosed nor rendered inherent" as the prior art did not disclose applying the compound to skin sunburn. *Perricone v. Medicis Pharmaceutical Corporation*, 432 F.3d 1368, 1378 (Fed. Cir. 2005). It is irrelevant that the prior art compound *may* have been applied to sunburned skin. *Id.* At 1379.

Because Xu et al. does not teach actual administration of the "modulator" nothing can occur in Xu et al., either literally or inherently.

7.4 XU ET AL. IS NOT AN ENABLING DISCLOSURE FOR THE ALLEGEDLY ANTICIPATORY SUBJECT MATTER

7.4.1. *Appellants' position that Xu et al. is not an enabling disclosure for what the Examiner asserts it teaches.*

Appellants note that for the anticipation rejection (either explicit or inherent) of the pending claims to be proper, Xu et al. must be enabling for what the Examiner alleged it teaches. Appellants further note that the following paragraph is the description in Xu et al. that the Examiner impliedly asserts is an enabling disclosure:

> In yet another aspect, the invention features a method for treating a subject having a metabolic disorder characterized by aberrant SARP3 polypeptide activity or aberrant SARP3 nucleic acid expression, e.g., obesity, diabetes, anorexia, or cachexia. The method includes administering to the subject a SARP3 modulator, e.g., in a pharmaceutically acceptable formulation or by using a gene therapy vector. Embodiments of this aspect of the invention include the SARP3 modulator being a small molecule, an anti-SARP3 antibody, a SARP3 polypeptide comprising the amino acid sequence of SEQ ID NO:2 or 5 or a fragment thereof, a SARP3 polypeptide comprising an amino acid sequence which is at least 90 percent identical to the amino acid sequence of SEQ ID NO:2 or 5, an isolated naturally occurring allelic variant of a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 or 5, an antisense SARP3 nucleic acid molecule, a nucleic acid molecule of SEQ ID NO: 1, 3, 4, or 6 or a fragment thereof, or a ribozyme.

Appellants note, however, that "mere naming or description of the subject matter is insufficient, if it cannot be produced without *undue experimentation*. Elan Pharm., Inc. v. Mayo Found. For Med. Educ. & Research, 346 F.3d 1051, 1054, 68

USPQ2d 1373, 1376 (Fed. Cir. 2003)", (emphasis added). M.P.E.P §2121.01. Appellants note that the claimed invention is based on the appellants' discovery of reduction in weight in two of the three independent lines of sFRP-5 transgenic mice, produced by appellants, which overexpress the sFRP-5 polypeptide (see page 21, lines 29 to 31), none of which is described in Xu et al. Appellants maintain that the minimal description in Xu et al. as relied upon for the anticipation rejection of the pending claims is insufficient to be an enabling disclosure, especially in light of the experimental results.

Furthermore, the same Examiner has previously acknowledged that the disclosure of Xu et al. is not enabling "for a method of modulating a SARP3 mediated lipid metabolism" (see Examiner's comments on page 6 of the Office Action issued May 16, 2006 (**Evidence Appendix Exhibit B.2**) in connection with U.S. Serial No. 10/338,604, of which the cited Xu et al. is the U.S. Patent Application Publication). More pertinently, on page 9 of the same document, the Examiner stated that to practice a method of modulating a SARP3-mediated activity comprising contacting a cell or tissue that expresses SARP3 with a SARP3 modulator that can modulate lipid metabolism "it would require *undue experimentation* by one of skill in the art to be able to practice the claimed invention" (emphasis added). Such a disclosure is thus clearly not enabling for modulating a SARP3-mediated activity.

"To serve as an anticipating reference, the reference must enable that which it is asserted to anticipate. A claimed invention cannot be anticipated by a prior art reference if the allegedly anticipatory disclosures cited as prior art are

not enabled" *Elan Pharmaceuticals, Inc. v. Mayo Foundation*, 346 F.3d 1051, 1054 (Fed. Cir. 2003) (internal citations omitted). A reference is enabling "if one of ordinary skill in the art could have combined the publication's description of the invention with his own knowledge to make the claimed invention." *In re Donohue*, 766 F.2d 531, 533 (Fed. Cir. 1985).

Courts have refused to find anticipation based on prior art that is not enabling. For instance, in *Rockwell Intern. Corp v. U.S.*, the Federal Circuit refused to invalidate patent claims based on prior art which did not "provide sufficient basic chemistry information to enable one skilled in the art to grow epitaxial . . . semiconductors" (i.e. practice the claimed process). *See Rockwell Intern. Corp v. U.S.*, 147 F.3d 1358, 1364 (Fed. Cir. 1998). Likewise, in *Application of Sheppard*, the Court of Customs and Patent Appeals held that a prior art reference which disclosed either the claimed compound *or* a different compound was not enabling and therefore could not anticipate the claim at issue. *Application of Sheppard*, 339 F.2d 238, 241-242 (C.C.P.A., 1964).

In the present case, the Examiner has cited prior art speculating on both the selection of a process and the result of such process. Such prior art, like the prior art in *Rockwell* and *Sheppard*, does not enable one skilled in the art to practice the claimed process. Accordingly, this prior art does not anticipate the claims in the present application.

Appellants maintain that the anticipation rejection, either express or inherent, based on Xu et al. is improper and must be withdrawn.

7.5 SUMMARY

For the foregoing reasons, Appellants submit that the Examiner's rejections of claims 1, 8-9 and 17-19 are erroneous, and respectfully submit that the rejections of these claims should be reversed.

Respectfully submitted,

Gary J. Gershik

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I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to:
Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450.

Gary J. Gershik 12/10/07

Gary J. Gershik Date
Reg. No. 39,992

CLAIMS APPENDIX

EXHIBIT A-1

In the Claims

Please replace the currently pending set of claims with the set of claims set forth below without prejudice under the provisions of 37 C.F.R. § 1.121:

1. (Currently Amended) A method of reducing the amount of adipose tissue in a subject comprising administering to the subject an amount of an sFRP-5 peptide effective to reduce the amount of adipose tissue, or an amount of a molecule effective to stimulate expression of the sFRP-5 peptide in the subject, wherein the sFRP-5 peptide comprises consecutive amino acids having the sequence set forth in SEQ ID NO: 1.

2-7. (Cancelled)

8. (Original) The method of claim 1, wherein the subject is human.

9. (Original) The method of claim 1, wherein the administration is parenteral, intradermal, transdermal, transmucosal, rectal, subcutaneous, or by inhalation.

10-16. (Cancelled)

17. (Original) A method of reducing the level of adipose tissue formation in a subject comprising administering to the subject an amount of an sFRP-5 peptide effective to reduce the level of adipose tissue formation, or an amount of a molecule effective to stimulate expression of the sFRP-5 peptide in the subject, wherein the sFRP-5 peptide comprises consecutive amino acids having the sequence set forth in SEQ ID NO: 1.

18. (New) The method of claim 17, wherein the subject is human.

19. (New) The method of claim 17, wherein the administration is parenteral, intradermal, transdermal, transmucosal, rectal, subcutaneous, or by inhalation.

EXHIBIT B

EVIDENCE APPENDIX

B.1	U.S. Patent Application Publication No. 2003/0143610 A1, published July 31, 2003
B.2	Office Action issued May 16, 2006 in connection with U.S. Serial No. 10/338,604
B.3	Statement of where B.1 and B.2 were entered into the record by the Examiner

EXHIBIT B-1

US 20030143610A1

(19) United States

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(54) METHODS FOR THE TREATMENT OF METABOLIC DISORDERS, INCLUDING OBESITY AND DIABETES

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(57) ABSTRACT

The invention relates to methods and compositions for the diagnosis and treatment of metabolic disorders, including, but not limited to, obesity, diabetes, overweight, insulin resistance, anorexia, and cachexia. The invention further provides methods for identifying a compound capable of treating a metabolic disorder. The invention also provides methods for identifying a compound capable of modulating a metabolic activity. Yet further, the invention provides a method for modulating a metabolic activity. In addition, the invention provides a method for treating a subject having a metabolic disorder characterized by aberrant SARP3 polypeptide activity or aberrant SARP3 nucleic acid expression. In another aspect, the invention provides methods for modulating lipogenesis in a subject and methods for modulating lipolysis in a subject.

METHODS FOR THE TREATMENT OF METABOLIC DISORDERS, INCLUDING OBESITY AND DIABETES

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 60/346,523, filed Jan. 8, 2002, the contents of which are incorporated herein by this reference.

BACKGROUND OF THE INVENTION

[0002] During normal embryonic and adult development of multicellular organisms, cells that are not necessary or deleterious are eliminated by a process referred to as programmed cell death or apoptosis (Ellis R. E. et al. (1991) *Annual Rev. Cell Biol.* 7:663-698). Programmed cell death occurs in both vertebrate and invertebrate species and is characterized by unique morphological alterations, such as cytoplasmic contraction and chromatin condensation, as well as by specific DNA cleavage into oligonucleosomal fragments. Unlike necrosis, programmed cell death or apoptosis is an irreversible process which in most systems appears to depend on the expression of a specific set of novel "death genes". Deregulation of this process contributes to the pathogenesis of several diseases including cancer, immunodeficiency, autoimmune diseases, and neurodegenerative disorders (Thompson C. B. et al. (1995) *Science* 267: 1456).

[0003] Recently, a new family of molecules believed to play a role in apoptosis has been identified (Melkonyan H. S. et al. (1997) *Proc. Natl. Acad. Sci.* 94:13636-13641). These molecules are referred to as Secreted Apoptosis-Related Proteins (SARP) and have a cysteine rich domain homologous to the cysteine rich domain of the frizzled proteins, a class of *D. melanogaster* integral membrane proteins which function as receptors for Wnt proteins, a family of highly conserved extracellular signaling molecules that regulate cell-to-cell interactions during embyogenesis, and have been implicated in cancer

[0004] Adipose tissue consists primarily of adipocytes. Vertebrates possess two distinct types of adipose tissue: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT stores and releases fat according to the nutritional needs of the animal. This stored fat is used by the body for (1) heat insulation (e.g., subcutaneous fat), (2) mechanical cushion (e.g., surrounding internal organs), and (3) as a source of energy. BAT burns fat, releasing the energy as heat through thermogenesis. BAT thermogenesis is used both (1) to maintain homeothermy by increasing thermogenesis in response to lower temperatures and (2) to maintain energy balance by increasing energy expenditure in response to increases in caloric intake (Sears, I. B. et al. (1996) *Mol. Cell. Biol.* 16(7):3410-3419). BAT is also the major site of thermogenesis in rodents and plays an important role in thermogenesis in human infants. In humans, and to a lesser extend rodents, brown fat diminishes with age, but can be re-activated under certain conditions, such as prolonged exposure to cold, maintenance on a high fat diet and in the presence of noradrenaline producing tumors.

[0005] Obesity represents the most prevalent of body weight disorders, affecting an estimated 30 to 50% of the middle-aged population in the western world. Other body weight disorders, such as anorexia nervosa and bulimia nervosa, which together affect approximately 0.2% of the female population of the western world, also pose serious health threats. Further, such disorders as anorexia and cachexia (wasting) are also prominent features of other diseases such as cancer, cystic fibrosis, and AIDS.

[0006] Obesity, defined as a body mass index (BMI) of 30 kg/m^2 or more, contributes to diseases such as coronary artery disease, hypertension, stroke, diabetes, hyperlipidemia and some cancers. (See, e.g., Nishina, P. M. et al. (1994), *Metab.* 43:554-558; Grundy, S. M. & Barnett, J. P. (1990), Dis. Mon. 36:641-731). Obesity is a complex multifactorial chronic disease that develops from an interaction of genotype and the environment and involves social, behavioral, cultural, physiological, metabolic and genetic factors.

[0007] Generally, obesity results when energy intake exceeds energy expenditure, resulting in the growth and/or formation of adipose tissue via hypertrophic and hyperplastic growth. Hypertrophic growth is an increase in size of adipocytes stimulated by lipid accumulation. Hyperplastic growth is defined as an increase in the number of adipocytes in adipose tissue. It is thought to occur primarily by mitosis of pre-existing adipocytes caused when adipocytes fill with lipid and reach a critical size. An increase in the number of adipocytes has far-reaching consequences for the treatment and prevention of obesity.

[0008] Diabetes mellitus is the most common metabolic disease worldwide. Every day, 1700 new cases of diabetes are diagnosed in the United States, and at least one-third of the 16 million Americans with diabetes are unaware of it. Diabetes is the leading cause of blindness, renal failure, and lower limb amputations in adults and is a major risk factor for cardiovascular disease and stroke.

[0009] Normal glucose homeostasis requires the finely tuned orchestration of insulin secretion by pancreatic beta cells in response to subtle changes in blood glucose levels, delicately balanced with secretion of counter-regulatory hormones such as glucagon. One of the fundamental actions of insulin is to stimulate uptake of glucose from the blood into tissues, especially muscle and fat. Type 1 diabetes results from autoimmune destruction of pancreatic beta cells causing insulin deficiency. Type 2 or non-insulin-dependent diabetes mellitus (NIDDM) accounts for >90% of cases and is characterized by a triad of (1) resistance to insulin action on glucose uptake in peripheral tissues, especially skeletal muscle and adipocytes, (2) impaired insulin action to inhibit hepatic glucose production, and (3) misregulated insulin secretion (DeFronzo, (1997) Diabetes Rev. 5:177-269). In most cases, type 2 diabetes is a polygenic disease with complex inheritance patterns (reviewed in Kahn et al., (1996) Annu. Rev. Med. 47:509-531).

[0010] Environmental factors, especially diet, physical activity, and age, interact with genetic predisposition to affect disease prevalence. Susceptibility to both insulin resistance and insulin secretory defects appears to be genetically determined (Kahn, et al.). Defects in insulin action precede the overt disease and are seen in non-diabetic relatives of diabetic subjects. In spite of intense investigation, the genes responsible for the common forms of Type 2 diabetes remain unknown.

DESCRIPTION OF THE INVENTION

[0011] The invention provides methods and compositions for the diagnosis and treatment of metabolic disorders, e.g.,

obesity, anorexia, cachexia, and diabetes. The invention is based, at least in part, on the discovery that SARP3 molecules, also referred to as SFRP5 molecules, are expressed at high levels in adipose tissue, e.g., white adipose tissue (WAT), and hypothalamus of normal mice. SARP3 molecules were further found to be upregulated in mice maintained on a high-fat diet and treated with leptin. Consistent with this observation, insulin-resistant ob/ob and db/db mice demonstrate a decrease in the expression level of SARP3. In human tissue samples, SARP3 is expressed in hypothalamus and WAT.

[0012] Accordingly, the invention provides methods for the diagnosis and treatment of metabolic disorders including, but not limited to, obesity, anorexia, cachexia, and diabetes, and disorders associated with an aberrant hypothalamus and adipocyte activity.

[0013] In one aspect, the invention provides methods for identifying a nucleic acid associated with a metabolic disorder, e.g., obesity, anorexia, cachexia, and diabetes. The method includes contacting a sample expressing a SARP3 nucleic acid or polypeptide with a test compound and assaying the ability of the test compound to modulate the expression of a SARP3 nucleic acid or the activity of a SARP3 polypeptide.

[0014] In another aspect, the invention provides methods for identifying a compound capable of treating a metabolic disorder, e.g., obesity, anorexia, cachexia, or diabetes. The method includes assaying the ability of the compound to modulate SARP3 nucleic acid expression or SARP3 polypeptide activity. In one embodiment, the ability of the compound to modulate SARP3 nucleic acid expression or SARP3 polypeptide activity is determined by detecting modulation of the level of β-catenin. In another embodiment, the ability of the compound to modulate SARP3 nucleic acid expression or SARP3 polypeptide activity is determined by detecting modulation of leptin or insulin sensitivity. In still another embodiment, the ability of the compound to modulate SARP3 nucleic acid expression or SARP3 polypeptide activity is determined by detecting modulation of food intake, body weight change, or glucose tolerance. In still another embodiment, the ability of the compound to modulate SARP3 nucleic acid expression or SARP3 polypeptide activity is determined by detecting modulation of hyperplastic growth of a SARP3 responsive cell or tissue. In another embodiment, the ability of the compound to modulate SARP3 nucleic acid expression or SARP3 polypeptide activity is determined by detecting modulation of cell differentiation of a SARP3 responsive cell or tissue. In yet another embodiment, the ability of the compound to modulate SARP3 nucleic acid expression or SARP3 polypeptide activity is determined by detecting modulation of hypertrophic growth of a SARP3 responsive cell or tissue.

[0015] In another aspect, the invention provides methods for identifying a compound capable of modulating an adipocyte activity, e.g., hyperplastic growth, hypertrophic growth, cell differentiation, or lipid metabolism (e.g., lipogenesis or lipolysis). The method includes contacting an adipocyte expressing a SARP3 nucleic acid or polypeptide with a test compound and assaying the ability of the test compound to modulate the expression of a SARP3 nucleic acid or the activity of a SARP3 polypeptide.

[0016] In another aspect, the invention provides methods for modulating an adipocyte activity, e.g., hyperplastic growth, hypertrophic growth, cell differentiation, or lipid metabolism. The method includes contacting an adipocyte with a SARP3 modulator (e.g., an anti-SARP3 antibody; a SARP3 polypeptide comprising the amino acid sequence of SEQ ID NO:2 or 5, or a fragment thereof; a SARP3 polypeptide comprising an amino acid sequence which is at least 90 percent identical to the amino acid sequence of SEQ ID NO:2 or 5; an isolated naturally occurring allelic variant of a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 or 5; a small molecule; an antisense SARP3 nucleic acid molecule; a nucleic acid molecule of SEQ ID NO: 1, 3, 4, or 6, or a fragment thereof; or a ribozyme) and determining the ability of the modulator to modify or alter an adipocyte activity.

[0017] In yet another aspect, the invention features a method for identifying a subject having a metabolic disorder characterized by aberrant SARP3 polypeptide activity or aberrant SARP3 nucleic acid expression, e.g., obesity, anorexia, cachexia, or diabetes. The method includes contacting a sample obtained from the subject, expressing a SARP3 nucleic acid or polypeptide with a test compound, and assaying the ability of the test compound to modulate the expression of a SARP3 nucleic acid or the activity of a SARP3 polypeptide.

[0018] In yet another aspect, the invention features a method for treating a subject having a metabolic disorder characterized by aberrant SARP3 polypeptide activity or aberrant SARP3 nucleic acid expression, e.g., obesity, diabetes, anorexia, or cachexia. The method includes administering to the subject a SARP3 modulator, e.g., in a pharmaceutically acceptable formulation or by using a gene therapy vector. Embodiments of this aspect of the invention include the SARP3 modulator being a small molecule, an anti-SARP3 antibody, a SARP3 polypeptide comprising the amino acid sequence of SEQ ID NO:2 or 5 or a fragment thereof, a SARP3 polypeptide comprising an amino acid sequence which is at least 90 percent identical to the amino acid sequence of SEQ ID NO:2 or 5, an isolated naturally occurring allelic variant of a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 or 5, an antisense SARP3 nucleic acid molecule, a nucleic acid molecule of SEQ ID NO: 1, 3, 4, or 6 or a fragment thereof, or a ribozyme.

[0019] Other features and advantages of the invention will be apparent from the following description and claims.

[0020] The invention provides methods and compositions for the diagnosis and treatment of a metabolic disorder, e.g., obesity, diabetes, anorexia, or cachexia. The invention is based, at least in part, on the discovery that the SARP3 (secreted apoptosis-related protein 3), also referred to herein as SFRP5 (secreted frizzled-related protein 5) nucleic acid and polypeptide molecules (e.g., GenBank Accession Nos. NM_003015 and AF117759) are expressed at high levels in adipose tissue and hypothalamus, and are downregulated in leptin pathway deficient genetic animal models of obesity, but upregulated when fed a high fat diet. Without intending to be limited to any particular theory or mechanism of action, it is believed that SARP3 molecules can modulate lipid metabolism.

[0021] As used herein, the term "metabolic disorder" includes a disorder, disease or condition which is caused or

characterized by an abnormal metabolism (i.e., the chemical changes in living cells by which energy is provided for vital processes and activities) in a subject. Metabolic disorders include diseases, disorders, or conditions associated with hyperglycemia or aberrant adipose cell (e.g., brown or white adipose cell) phenotype or function. Metabolic disorders can be characterized by a misregulation (e.g., an aberrant down-regulation or upregulation) of SARP3 activity. Metabolic disorders can detrimentally affect cellular functions such as cellular proliferation, growth, differentiation, or migration, cellular regulation of homeostasis, inter- or intra-cellular communication; tissue function, such as liver function, renal function, or adipocyte function; systemic responses in an organism, such as hormonal responses (e.g., insulin response). Examples of metabolic disorders include obesity, diabetes, hyperphagia, endocrine abnormalities, triglyceride storage disease, Bardet-Biedl syndrome, Lawrence-Moon syndrome, Prader-Labhart-Willi syndrome, anorexia, cachexia, and disorders of lipid metabolism.

[0022] Obesity is defined as a body mass index (BMI) of 30 kg/m^2 or more (National Institute of Health, Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults (1998)). However, the invention is also intended to include a disease, disorder, or condition that is characterized by a body mass index (BMI) of 25 kg/m^2 or more, 26 kg/m^2 or more, 27 kg/m^2 or more, 28 kg/m^2 or more, 29 kg/m^2 or more, 29.5 kg/m^2 or more, or 29.9 kg/m^2 or more, all of which are typically referred to as overweight (National Institute of Health, Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults (1998)).

[0023] "Subject", as used herein, can refer to a mammal, e.g., a human, or to an experimental or naturally occurring animal disease model. The subject can also be a non-human animal, e.g., a horse, cow, goat, or other domestic animal. A subject, e.g., a human subject, can also be a patient, i.e., an individual receiving medical attention, care, or treatment.

[0024] As used interchangeably herein, "SARP3 activity, ""biological activity of SARP3" or "functional activity of SARP3," includes an activity exerted by a SARP3 protein, polypeptide or nucleic acid molecule on a SARP3 responsive cell or tissue, e.g., adipocytes, or on a SARP3 protein substrate, e.g., a Wnt protein, as determined in vivo, or in vitro, according to standard techniques. SARP3 activity can be a direct activity, such as an association with a SARP3 target molecule. As used herein, a "substrate" or "target molecule" or "binding partner" is a molecule with which a SARP3 protein binds or interacts in nature, such that a SARP3 mediated function, e.g., modulation of a metabolic activity, is achieved. A SARP3 target molecule can be a non-SARP3 molecule or a SARP3 protein or polypeptide. Examples of such target molecules include proteins in the same metabolic pathway as the SARP3 protein, e.g., proteins which may function upstream (including both stimulators and inhibitors of activity) or downstream of the SARP3 protein in a pathway involving regulation of metabolism. Alternatively, a SARP3 activity is an indirect activity, such as a cellular signaling activity mediated as a consequence of the interaction of the SARP3 protein with a SARP3 target molecule.

[0025] The biological activities of SARP3 are described herein. For example, the SARP3 proteins can have one or more of the following activities: (1) the ability to interact with a non-SARP molecule on the surface of the same cell which expresses it; (2) the ability to interact with a non-SARP molecule on the surface of a different cell; (3) the ability to activate a SARP-dependent signal transduction pathway, e.g., a pathway involving Wnt protein signaling; (4) the abilitity to modulate programmed cell death; (5) the ability to modulate β-catenin gene expression or protein activity; (6) the ability to modulate glucose metabolism, e.g., glucose secretion or uptake; (7) the ability to modulate insulin metabolism, e.g., insulin secretion or uptake; or (8) the ability to modulate lipid metabolism, e.g., lipogenesis or lipolysis. Thus, the SARP3 proteins can be used to, for example, (1) modulate the interaction with a non-SARP molecule on the surface of the same cell which expresses it; (2) modulate the interaction with a non-SARP molecule on the surface of a different cell; (3) activate a SARP-dependent signal transduction pathway; e.g., a pathway involving Wnt protein signaling; (4) modulate programmed cell death; (5) modulate β-catenin gene expression or protein activity; (6) modulate glucose metabolism, e.g., glucose secretion or uptake; (7) modulate insulin metabolism, e.g., insulin secretion or metabolism; or (8) modulate lipid metabolism, e.g., lipogenesis or lipolysis.

[0026] As used herein, "metabolic activity" includes an activity exerted by an adipose cell, or an activity that takes place in an adipose cell. For example, such activities include cellular processes that contribute to the physiological role of adipose cells, such as lipogenesis and lipolysis, insulin metabolism, glucose metabolism, and include, but are not limited to, cell proliferation, differentiation, growth, migration, programmed cell death, uncoupled mitochondrial respiration, and thermogenesis. A metabolic activity of an adipose cell also includes secondary effects on processes in cells in other tissues, e.g., liver, skeletal muscle, including cardiac muscle, and kidney.

[0027] Various aspects of the invention are described in further detail in the following subsections:

[0028] I. Screening Assays

[0029] The invention provides methods (also referred to herein as a "screening assays") for identifying modulators, i.e., candidate or test compounds or agents (e.g., peptides, peptidomimetics, small molecules or other drugs) which bind to SARP3 polypeptides, have a stimulatory or inhibitory effect on, for example, SARP3 expression or SARP3 activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of a SARP3 substrate.

[0030] In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of a SARP3 polypeptide or polypeptide or biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a SARP3 polypeptide, or biologically active portion thereof. The test compounds of the invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; 'one-bead one-compound' library methods; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide librar-

ies, while the other four approaches are applicable to peptide, non-peptide oligomer, and small molecule libraries of compounds (Lam, K. S. (1997) *Anticancer Drug Des.* 12:145).

[0031] Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt et al. (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann et al. (1994). *J. Med. Chem.* 37:2678; Cho et al. (1993) *Science* 261:1303; Carrell et al. (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell et al. (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop et al. (1994) *J. Med. Chem.* 37:1233.

[0032] Libraries of compounds may be presented in solution (e.g., Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (U.S. Pat. No. 5,223,409), spores (U.S. Pat. No. 5,223,409), plasmids (Cull et al. (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or phage (Scott and Smith (1990) *Science* 249:386-390; Devlin (1990) *Science* 249:404-406; Cwirla et al. (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382; Felici (1991) *J. Mol. Biol.* 222:301-310); Ladner supra.).

[0033] In one embodiment, an assay is a cell-based assay in which a cell which expresses a SARP3 polypeptide, or biologically active portion thereof, is contacted with a test compound and the ability of the test compound to modulate SARP3 activity is determined. Determining the ability of the test compound to modulate SARP3 activity can be accomplished by monitoring, for example, a modulation of the intracellular level of β-catenin, a modulation in Wnt signaling, a modulation in glucose concentration, a modulation in glucose uptake, a modulation in secretion of glycerol, insulin, or glucagon. The cell, for example, can be of mammalian origin, e.g., a kidney cell, a spleen cell, or a fat cell, such as an adipocyte.

[0034] In an embodiment, an assay is a cell-based assay in which a cell which expresses a constitutively active SARP3 polypeptide, or a constitutively active portion thereof, is contacted with a test compound and the ability of the test compound to inhibit SARP3 activity is determined.

[0035] In a preferred embodiment, an assay is a cell-based assay in which a cell, e.g., a *Xenopus oocyte*, which expresses a constitutively active SARP3 polypeptide, or a constitutively active portion thereof, is contacted with a test compound, and the ability of the test compound to modulate Wnt signaling is determined.

[0036] The ability of the test compound to modulate SARP3 binding to a substrate, e.g., a Wnt protein, or to bind SARP3 itself can also be determined. Determining the ability of the test compound to modulate SARP3 binding to a substrate can be accomplished, for example, by coupling the SARP3 substrate, e.g., a Wnt protein, with a radioisotope, an enzymatic label, or a fluorescent label such that binding of the SARP3 substrate to SARP3 can be determined by detecting the labeled SARP3 substrate in a complex. Alternatively, SARP3 can be coupled with a radioisotope, an enzymatic label, or a fluorescent label to monitor the ability of a test compound to modulate SARP3 binding to a SARP3 substrate in a complex. Determining the ability of the test compound to bind SARP3 can be accomplished, for example, by coupling the compound with a radioisotope, an enzymatic label, ora fluorescent label such that binding of the compound to SARP3 can be determined by detecting the labeled SARP3 compound in a complex. For example, compounds (e.g., SARP3 substrates) can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. Compounds can be fluorescently labeled with, for example, fluorescein, rhodamine, AMCA, or TRF, and the fluorescent label detected by exposure of the compound to a specific wavelength of light.

[0037] It is also within the scope of this invention to determine the ability of a compound (e.g., a SARP3 substrate, e.g., a Wnt protein) to interact with SARP3 without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of a compound with SARP3 without the labeling of either the compound or SARP3. (See McConnell, H. M. et al. (1992) *Science* 257:1906-1912.) As used herein, a "microphysiometer" (e.g., the Cytosensor® Microphysiometer System by Molecular Devices Corp., Sunnyvale Calif.) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and SARP3.

[0038] In another embodiment, an assay is a cell-based assay comprising contacting a cell which expresses a SARP3 polypeptide or SARP3 target molecule (e.g., a SARP3 substrate, e.g., a Wnt protein) with a test compound and determining the ability of the test compound to modulate (e.g., stimulate or inhibit) the activity of the SARP3 target molecule, e.g., a Wnt signaling pathway. Determining the ability of the test compound to modulate the activity of a SARP3 target molecule can be accomplished, for example, by determining the ability of the SARP3 polypeptide to bind to or interact with the SARP3 target molecule in the presence of the test compound, or by determining the ability of the SARP3 polypeptide to bind to or interact with the SARP3 target molecule before or after exposure of the SARP3 target molecule with the test compound.

[0039] Determining the ability of the SARP3 polypeptide, or a biologically active fragment thereof, to bind to or interact with a SARP3 target molecule can be accomplished by any one of the methods described above for determining direct binding. In a preferred embodiment, determining the ability of the SARP3 polypeptide to bind or interact with a SARP3 target molecule, e.g., a Wnt protein, can be accomplished by determining a change in the biological or chemical activity of the resulting from the binding or interaction of the SARP3 target molecule with the SARP3 polypeptide. For example, the activity of the target molecule can be determined by detecting an enzymatic or catalytic activity of the target using an appropriate substrate, by detecting the induction of a reporter gene (comprising a target-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, e.g., luciferase), or by detecting a target-regulated cellular response.

[0040] In yet another embodiment, an assay of the invention is a cell-free assay in which a SARP3 polypeptide or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the SARP3 polypeptide or biologically active portion thereof is determined. Preferred biologically active portions of the SARP3 polypeptides to be used in any of the assays of the invention include fragments which participate in interactions with non-SARP3 molecules, e.g., fragments with high surface probability scores. Binding of the test compound to the SARP3 polypeptide can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting the SARP3 polypeptide, or biologically active portion thereof, with a known compound which binds SARP3 to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a SARP3 polypeptide, wherein determining the ability of the test compound to interact with a SARP3 polypeptide comprises determining the ability of the test compound to preferentially bind to SARP3, or biologically active portion thereof, as compared to the known compound.

[0041] In another embodiment, the assay is a cell-free assay in which a SARP3 polypeptide, or biologically active portion thereof, is contacted with a test compound and the ability of the test compound to modulate (e.g., stimulate or inhibit) the activity of the SARP3 polypeptide, or biologically active portion thereof, is determined. Determining the ability of the test compound to modulate the activity of a SARP3 polypeptide can be accomplished, for example, by determining the ability of the SARP3 polypeptide to bind to or interact with a SARP3 target molecule by any of the methods described above for determining direct binding. Determining the ability of the SARP3 polypeptide to bind to a SARP3 target molecule can also be accomplished using a technology such as real-time Biomolecular Interaction Analysis (BIA). (See, e.g., Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo et al. (1995) *Curr. Opin. Struct. Biol.* 5:699-705.) As used herein, "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (e.g., BIAcore). Changes in the optical phenomenon of surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

[0042] In an alternative embodiment, determining the ability of the test compound to modulate the activity of a SARP3 polypeptide can be accomplished by determining the ability of the SARP3 polypeptide to further modulate the activity of a downstream or upstream effector of a SARP3 target molecule. For example, the activity of the effector molecule on an appropriate target can be determined or the binding of the effector to an appropriate target can be determined, as previously described.

[0043] In yet another embodiment, determining the ability of the test compound to modulate the activity of a SARP3 polypeptide can be accomplished by determining the ability of the test compound to modulate the activity of a SARP3 target molecule, e.g., a SARP3 substrate, e.g., Wnt protein. In a preferred embodiment, the assay includes contacting the SARP3 polypeptide, or biologically active portion thereof, with a known compound which binds SARP3, e.g., a SARP3 substrate, to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the known compound, wherein determining the ability of the test compound to interact with the known compound includes determining the ability of the test compound to preferentially bind to the known compound, or biologically active portion thereof, as compared to the SARP3 polypeptide.

[0044] In yet another embodiment, the cell-free assay involves contacting a SARP3 polypeptide, or biologically active portion thereof, with a known compound which binds the SARP3 polypeptide to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the SARP3 polypeptide, wherein determining the ability of the test compound to interact with the SARP3 polypeptide comprises determining the ability of the SARP3 polypeptide to preferentially bind to or modulate the activity of a SARP3 target molecule as compared to the known compound.

[0045] In one or more embodiments of the above assay methods of the invention, it may be desirable to immobilize either SARP3 or its target molecule to facilitate separation of complexed from uncomplexed forms of SARP3 and its target molecule, as well as to accommodate automation of the assay. Binding of a test compound to a SARP3 polypeptide, or interaction of a SARP3 polypeptide with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/SARP3 fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.) or glutathione derivatized micrometer plates, which can then combined with the test compound or the test compound and either the non-adsorbed target protein or SARP3 polypeptide, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or micrometer plates are washed to remove any unbound components, the matrix immobilized in the case of beads, and the presence of complex is then determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of SARP3 binding or activity determined using standard techniques.

[0046] Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either a SARP3 polypeptide or a SARP3 target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated SARP3 polypeptide or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (e.g., a biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96-well microtiter plates (Pierce Chemicals). Alternatively, antibodies reactive with SARP3 polypeptide or its target molecules, but which do not interfere with binding of the SARP3 polypeptide to its target molecule can be derivatized to the wells of the plate, such that complexes of target bound to SARP3 polypeptide will be trapped in the wells by the antibody. Methods for detecting such complexes, in addition to those described above for the GST-immobilized com-

plexes, include immunodetection of complexes using antibodies reactive with the SARP3 polypeptide or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the SARP3 polypeptide or target molecule.

[0047] In another embodiment, modulators of SARP3 expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of SARP3 mRNA or polypeptide in the cell is determined. The level of expression of SARP3 mRNA or polypeptide in the presence of the candidate compound is compared to the level of expression of SARP3 mRNA or polypeptide in the absence of the candidate compound. The candidate compound can then be identified as a modulator of SARP3 expression based on this comparison. For example, when expression of SARP3 mRNA or polypeptide is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of SARP3 mRNA or polypeptide expression. Alternatively, when expression of SARP3 mRNA or polypeptide is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of SARP3 mRNA or polypeptide expression. The level of SARP3 mRNA or polypeptide expression in the cells can be determined by methods described herein for detecting SARP3 mRNA or polypeptide.

[0048] In yet another aspect of the invention, the SARP3 polypeptides can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, e.g., U.S. Pat. No. 5,283,317; Zervos et al. (1993) *Cell* 72:223-232; Madura et al. (1993) *J. Biol. Chem.* 268:12046-12054; Bartel et al. (1993) *Biotechniques* 14:920-924; Iwabuchi et al. (1993) *Oncogene* 8:1693-1696; and Brent WO 94/10300), to identify other proteins which bind to or interact with SARP3 (e.g., "SARP3-binding proteins" or "SARP3-bp") and are involved in SARP3 activity. Such SARP3-binding proteins are also likely to be involved in the propagation of pathway signals mediated by the SARP3 polypeptides or SARP3 targets as, for example, upstream or downstream elements of a SARP3-mediated signaling pathway. If there is an enhancement or stimulation of a SARP3-mediated signaling pathway, the SARP3-binding proteins are likely to be SARP3 stimulators. Alternatively, if there is a reduction of a SARP3-mediated signaling pathway, the SARP3-binding proteins are likely to be SARP3 inhibitors.

[0049] The two-hybrid, or "bait and prey", system is based on the modular nature of most transcription factors which consist of separable DNA-binding and activation domains. This enables an assay that utilizes two different DNA constructs. Briefly, one construct containing a gene sequence that encodes a SARP3 polypeptide ("bait protein") is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences that encodes an unidentified protein (i.e., the "prey" or "sample"), is fused to a gene that encodes the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact in vivo to form a complex of the SARP3 and the target molecule, the DNA-binding and activation domains of the transcription factor will be brought into close proximity to form a functional transcription factor. A reporter gene (e.g., LacZ) operably linked to a transcriptional regulatory site responsive to the transcription factor will then be transcribed. Detection of the expression of the reporter gene enables the identification and isolation of cell colonies containing the functional transcription factor. Subsequently, these cell colonies can then be used to clone and identify the sequence of the "bait" protein.

[0050] The ability of a test compound to modulate insulin sensitivity of a cell can be determined by performing an assay in which cells, e.g., adipose cells, are contacted with the test compound, e.g., transformed to express the test compound; incubated with radioactively labeled glucose (e.g., ^{14}C-glucose); and treated with insulin. An increase or decrease in the amount of glucose in cells that contain the test compound, relative to cells that do not contain the test compound indicates that the test compound can modulate insulin sensitivity of the cells. Alternatively, cells that contain the test compound can be incubated with a radioactively labeled phosphate source (e.g., ^{32}P-ATP) and treated with insulin. Phosphorylation of proteins in the insulin pathway, e.g., the insulin receptor, can then be measured. An increase or decrease in phosphorylation of a protein in the insulin pathway in cells containing the test compound, relative to cells that do not contain the test compound indicates that the test compound can modulate insulin sensitivity of the cells.

[0051] In another aspect, the invention pertains to a combination of two or more of the assays described herein. For example, a modulating agent can be identified using a cell-based or a cell-free assay, and the ability of the agent to modulate the activity of a SARP3 protein can be confirmed in vivo, e.g., in an animal such as an animal model for obesity, diabetes, anorexia, or cachexia. Examples of animals that can be used include the transgenic mouse described in U.S. Pat. No. 5,932,779 that contains a mutation in an endogenous melanocortin-4-receptor (MC4-R) gene; animals having mutations which lead to syndromes that include obesity symptoms (described in, for example, Friedman, J. M. et al. (1991) *Mamm. Gen.* 1:130-144; Friedman, J. M. and Liebel, R. L. (1992) *Cell* 69:217-220; Bray, G. A. (1992) *Prog. Brain Res.* 93:333-341; and Bray, G. A. (1989) *Amer. J. Clin. Nutr.* 5:891-902); the mice with a diabetes mutation (db) which is attributed to a mutation in the leptin receptor gene ($Lepr^{db}$; described in, for example, Chen, H. et al. (1996) *Cell* 84:491-5; Chua S C Jr et al. (1996) *Science* 271:994-6; and Lee, G. H. et al (1996) *Nature* 379:632-5); the mice homozygous for the obese (ob) mutation (described in, for example, MacDougald, O. A. et al (1995) *Proc. Natl. Acad. Sci. USA* 92:9034-7); the animals described in Stubdal H. et al. (2000) *Mol. Cell Biol.* 20(3):878-82 (the mouse tubby phenotype characterized by maturity-onset obesity); the animals described in Abadie J. M. et al. *Lipids* (2000) 35(6):613-20 (the obese Zucker rat (ZR), a genetic model of human youth-onset obesity and type 2 diabetes mellitus); the animals described in Shaughnessy S. et al. (2000) *Diabetes* 49(6):904-11 (mice null for the adipocyte fatty acid binding protein); the animals described in Loskutoff D. J. et al. (2000) *Ann. N.Y. Acad. Sci.* 902:272-81 (the fat mouse); or animals having mutations which lead to syndromes that include diabetes (described in, for example, Alleva et al. (2001) *J. Clin. Invest.* 107:173-180; Arakawa et al. (2001) *Br. J. Pharmacol.* 132:578-586; Nakamura et al. (2001) *Diabetes Res. Clin. Pract.* 51:9-20; O'Harte et al. (2001) *Regul. Pept.* 96:95-104; Yamanouchi et al. (2000) *Exp. Anim.* 49:259-266; Hoenig et al. (2000) *Am. J. Pathol.* 157:2143-2150; Reed et al. (2000) *Metabolism*

49:1390-1394; and Clark et al. (2000) *J. Pharmacol. Toxicol. Methods* 43: 1-10). Other examples of animals that may be used include non-recombinant, non-genetic animal models of obesity such as, for example, rabbit, mouse, or rat models in which the animal has been exposed to either prolonged cold, thereby, inducing hypertrophy of BAT and increasing BAT thermogenesis (Himms-Hagen, J. (1990), supra). Alternatively, another non-genetic animal model of obesity can involve diet-induced obesity, e.g., by long-term overfeeding or feeding on a high fat diet.

[0052] In another aspect, the invention pertains to computer modeling and searching technologies to identify compounds, or improve previously identified compounds, that can modulate SARP3 gene expression or protein activity. Having identified such a compound or composition enables identification of active sites or regions, as well as other sites or regions critical in the function of the protein. Such active sites are often ligand, e.g., substrate, binding sites. The active site can be identified using methods known in the art including, for example, from the amino acid sequences of peptides, from the nucleotide sequences of nucleic acids, or from studies of complexes of the relevant compound or composition with its natural ligand. In the latter case, chemical or X-ray crystallographic methods are useful in identifying residues in the active site by locating the position of the complexed ligand.

[0053] The three dimensional geometric structure of the active site can be determined using known methods, including X-ray crystallography, from which spatial details of the molecular structure can be obtained. Additionally, solid or liquid phase NMR can be used to determine certain intramolecular distances. Any other experimental method of structure determination known in the art can be used to obtain partial or complete geometric structures. The geometric structures measured with a complexed ligand, natural or artificial, can increase the accuracy of the active site structure determined.

[0054] When only an incomplete or insufficiently accurate structure is determined, methods of computer based numerical modeling can be used to complete or improve the accuracy of the structure. Any recognized modeling method may be used, including parameterized models specific to particular biopolymers, such as proteins or nucleic acids, molecular dynamics models based on computing molecular motions, statistical mechanics models based on thermal ensembles, or combined models. For most types of models, standard molecular force fields, which include the forces between constituent atoms and groups, are necessary, and can be selected from force fields known in physical chemistry. The incomplete or less accurate experimental structures can serve as constraints on the complete and more accurate structures computed by these modeling methods.

[0055] Having determined the structure of the active site, either experimentally, by modeling, or by a combination of approaches, candidate modulating compounds can be identified by searching databases containing compounds along with information on their molecular structure. Such searches seek compounds having structures that match the determined active site structure and that interact with the groups defining the active site. Such a search can be manual, but is preferably computer assisted. Compounds identified using these search methods can be tested in any of the screening assays described herein to verify their ability to modulate SARP3 activity.

[0056] Alternatively, these methods can be used to identify improved modulating compounds from an already known modulating compound or ligand. The composition of the known compound can be modified and the structural effects of the modification can be determined by applying the experimental and computer modeling methods described above to the new composition. The altered structure is then compared to the active site structure of the compound to determine if an improved fit or interaction results. In this manner, systematic variations in composition, such as by varying side groups, can be quickly evaluated to obtain modified modulating compounds or ligands with improved specificity or activity.

[0057] Kaul ((1998) *Prog. Drug Res.* 50:9-105) provides a review of modeling techniques for the design of receptor ligands and drugs. Computer programs that screen and graphically depict chemicals are available from companies such as BioDesign, Inc. (Pasadena, Calif.), Oxford Molecular Design (Oxford, UK), and Hypercube, Inc. (Cambridge, Ontario).

[0058] Although described above with reference to design and generation of compounds which can alter the ability of SARP3 to bind its target molecule, e.g., a substrate, one can also screen libraries of known compounds, including natural products or synthetic chemicals, and biologically active materials, including proteins, for compounds which are inhibitors or activators.

[0059] This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein in an appropriate animal model, e.g., animal models for obesity, diabetes, cachexia, or anorexia.

[0060] In addition, transgenic animals that express a human SARP3 can be used to confirm the in vivo effects of a modulator of SARP3 identified by a cell-based or cell-free screening assay described herein. Animals of any non-human species, including, but not limited to, mice, rats, rabbits, guinea pigs, pigs, micro-pigs, goats, and non-human primates, e.g., baboons, monkeys, and chimpanzees, may be used to generate SARP3 transgenic animals. Alternatively, the transgenic animal comprises a cell, or cells, that includes a gene which misexpresses an endogenous SARP3 orthologue such that expression is disrupted, e.g., a knockout animal. Such animals are also useful as a model for studying the disorders which are related to mutated or misexpressed SARP3 alleles.

[0061] Any technique known in the art may be used to introduce the human SARP3 transgene into non-human animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (U.S. Pat. No. 4,873,191); retrovirus mediated gene transfer into germ lines (Van der Putten et al. (1985) *Proc. Natl. Acad. Sci. USA* 82:6148-6152); gene targeting in embryonic stem cells (Thompson et al. (1989) *Cell* 56:313-321); electroporation of embryos (Lo (1983) *Mol Cell. Biol.* 3:1803-1814); and sperm-mediated gene transfer (Lavitrano et al. (1989) *Cell* 57:717-723). For a

review of such techniques, see Gordon (1989) Transgenic Animals, *Intl. Rev. Cytol.* 115:171-229, which is incorporated by reference herein in its entirety.

[0062] The invention provides for transgenic animals that carry the SARP3 transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, i.e., mosaic animals. The transgene may be integrated as a single transgene or in concatamers, e.g., head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. ((1992) *Proc. Natl. Acad. Sci. USA* 89: 6232-6236). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest and will be apparent to those of skill in the art. When it is desired that the SARP3 transgene be integrated into the chromosomal site of the endogenous SARP3 gene, gene targeting is preferred. Briefly, this technique employs vectors that contain nucleotide sequences homologous to the endogenous SARP3 gene and/or sequences flanking the gene. The vectors are designed to integrate into the chromosomal site of the endogenous SARP3 gene, thereby disrupting the expression of the endogenous gene. The transgene may also be selectively expressed in a particular cell type with concomitant inactivation of the endogenous SARP3 gene in only that cell type, by following, for example, the teaching of Gu et al. ((1994) *Science* 265:103-106). The regulatory sequences required for such a cell-type specific recombination will depend upon the particular cell type of interest and will be apparent to those of skill in the art.

[0063] Once founder animals have been generated, standard analytical techniques such as Southern blot analysis or PCR techniques are used to analyze animal tissues to determine whether integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the founder animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, in situ hybridization analysis, and RT-PCR. Samples of SARP3 gene-expressing tissue, may also be evaluated immunocytochemically using antibodies specific for the SARP3 transgene product.

[0064] An agent identified as described herein (e.g., a SARP3 modulating agent, an antisense SARP3 nucleic acid molecule, a SARP3-specific antibody, or a SARP3-binding partner) can be used in an animal model described above to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

[0065] II. Predictive Medicine:

[0066] The invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the invention relates to diagnostic assays for detecting SARP3 polypeptide and/or nucleic acid expression as well as determining SARP3 activity, in the context of a biological sample (e.g., blood, serum, cells, or tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant or unwanted SARP3 expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with SARP3 polypeptide activity or nucleic acid expression. For example, mutations in a SARP3 gene can be assayed in a biological sample. Such assays can be used for a prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with SARP3 polypeptide activity or nucleic acid expression.

[0067] Another aspect of the invention pertains to monitoring the influence of agents (e.g., drugs, compounds) on the expression or activity of SARP3 in clinical trials.

[0068] These and other agents are described in further detail in the following sections.

[0069] A. Diagnostic Assays For Metabolic Disorders

[0070] An exemplary method for detecting the presence or absence of SARP3 polypeptide or nucleic acid in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting SARP3 polypeptide or nucleic acid (e.g., mRNA, or genomic DNA) that encodes SARP3 polypeptide, such that the presence of SARP3 polypeptide or nucleic acid is detected in the biological sample. In another aspect, the invention provides a method for detecting the presence of SARP3 activity in a biological sample by contacting the biological sample with an agent capable of detecting an indicator of SARP3 activity such that the presence of SARP3 activity is detected in the biological sample. A preferred agent for detecting SARP3 mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to SARP3 mRNA or genomic DNA. The nucleic acid probe can be, for example, the SARP3 nucleic acid set forth in SEQ ID NO: 1, 3, 4, or 6, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to SARP3 mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

[0071] A preferred agent for detecting SARP3 polypeptide is an antibody capable of binding to SARP3 polypeptide, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or $F(ab')_2$) can be used. The term "label", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin.

[0072] The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That

is, the detection method of the invention can be used to detect SARP3 mRNA, polypeptide, or genomic DNA in a biological sample in vitro as well as in vivo. For example, in vitro techniques for detection of SARP3 mRNA include Northern hybridizations, in situ hybridizations, RT-PCR, and Taqman analyses. In vitro techniques for detection of SARP3 polypeptide include enzyme linked immunosorbent assays (ELISAs), western blots, immunoprecipitations and immunofluorescence. In vitro techniques for detection of SARP3 genomic DNA include Southern hybridizations. Furthermore, in vivo techniques for detection of SARP3 polypeptide include introducing into a subject a labeled anti-SARP3 antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

[0073] The invention also provides diagnostic assays for identifying the presence or absence of a genetic alteration characterized by at least one of: (i) aberrant modification or mutation of a gene encoding a SARP3 polypeptide; (ii) aberrant expression of a gene encoding a SARP3 polypeptide; (iii) mis-regulation of the gene; or (iv) aberrant post-translational modification of a SARP3 polypeptide, wherein a wild-type form of the gene encodes a polypeptide with a SARP3 activity. "Misexpression or aberrant expression", as used herein, refers to a non-wild type pattern of gene expression, at the RNA or protein level. It includes, but is not limited to, expression at non-wild type levels (e.g., over or under expression); a pattern of expression that differs from wild type in terms of the time or stage at which the gene is expressed (e.g., increased or decreased expression (as compared with wild type) at a predetermined developmental period or stage); a pattern of expression that differs from wild type in terms of decreased expression (as compared with wild type) in a predetermined cell type or tissue type; a pattern of expression that differs from wild type in terms of the splicing size, amino acid sequence, post-transitional modification, or biological activity of the expressed polypeptide; a pattern of expression that differs from wild type in terms of the effect of an environmental stimulus or extracellular stimulus on expression of the gene (e.g., a pattern of increased or decreased expression (as compared with wild type) in the presence of an increase or decrease in the strength of the stimulus).

[0074] In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a serum sample isolated by conventional means from a subject.

[0075] In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting SARP3 polypeptide, mRNA, or genomic DNA, such that the presence of SARP3 polypeptide, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of SARP3 polypeptide, mRNA or genomic DNA in the control sample with the presence of SARP3 polypeptide, mRNA or genomic DNA in the test sample.

[0076] The invention also encompasses kits for detecting the presence of SARP3 in a biological sample. For example, the kit can comprise a labeled compound or agent capable of detecting SARP3 polypeptide or mRNA in a biological sample; means for determining the amount of SARP3 in the sample; and means for comparing the amount of SARP3 in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect SARP3 polypeptide or nucleic acid.

[0077] B. Prognostic Assays For Metabolic Disorders

[0078] The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant or unwanted SARP3 expression or activity. As used herein, the term "aberrant" includes a SARP3 expression or activity which deviates from the wild type SARP3 expression or activity. Aberrant expression or activity includes increased or decreased expression or activity, as well as expression or activity which does not follow the wild type developmental pattern of expression or the subcellular pattern of expression. For example, aberrant SARP3 expression or activity is intended to include the cases in which a mutation in the SARP3 gene causes the SARP3 gene to be under-expressed or over-expressed and situations in which such mutations result in a non-functional SARP3 polypeptide or a polypeptide which does not function in a wild-type fashion, e.g., a polypeptide which does not interact with a SARP3 substrate, e.g., □-catenin, or one which interacts with a non-SARP3 substrate. As used herein, the term "unwanted" includes an unwanted phenomenon involved in a biological response, such as cellular proliferation. For example, the term "unwanted" includes a SARP3 expression or activity which is undesirable in a subject.

[0079] The assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having, or at risk of developing, a disorder associated with a misregulation in SARP3 polypeptide activity or nucleic acid expression, such as a metabolic disorder. Alternatively, the prognostic assays can be utilized to identify a subject having, or at risk for developing, a disorder associated with a misregulation in SARP3 polypeptide activity or nucleic acid expression, such as a metabolic disorder. Thus, the invention provides a method for identifying a disease or disorder associated with aberrant or unwanted SARP3 expression or activity in which a test sample is obtained from a subject and SARP3 polypeptide or nucleic acid (e.g., mRNA or genomic DNA) is detected, wherein the presence of SARP3 polypeptide or nucleic acid is diagnostic for a subject having, or at risk of developing, a disease or disorder associated with aberrant or unwanted SARP3 expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (e.g., serum), cell sample, or tissue.

[0080] Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (e.g., an activator, inhibitor, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant or unwanted SARP3 expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a metabolic disorder. Thus, the invention provides methods

for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant or unwanted SARP3 expression or activity in which a test sample is obtained and SARP3 polypeptide or nucleic acid expression or activity is detected (e.g., wherein the abundance of SARP3 polypeptide or nucleic acid expression or activity is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant or unwanted SARP3 expression or activity).

[0081] The methods of the invention can also be used to detect genetic alterations in a SARP3 gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by misregulation in SARP3 polypeptide activity or nucleic acid expression, such as a metabolic disorder. In preferred embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding a SARP3-polypeptide, or the mis-expression of the SARP3 gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of: (1) a deletion of one or more nucleotides from a SARP3 gene; (2) an addition of one or more nucleotides to a SARP3 gene; (3) a substitution of one or more nucleotides of a SARP3 gene; (4) a chromosomal rearrangement of a SARP3 gene; (5) an alteration in the level of a messenger RNA transcript of a SARP3 gene; (6) aberrant modification of a SARP3 gene, such as of the methylation pattern of the genomic DNA; (7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a SARP3 gene; (8) a non-wild type level of a SARP3-polypeptide; (9) allelic loss of a SARP3 gene; and (10) inappropriate post-translational modification of a SARP3 polypeptide. As described herein, there are a large number of assays known in the art which can be used for detecting alterations in a SARP3 gene. A preferred biological sample is a tissue or serum sample isolated, e.g., by conventional means, from a subject.

[0082] In certain embodiments, detection of the alteration involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, e.g., U.S. Pat. Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, e.g., Landegran et al. (1988) *Science* 241:1077-1080; and Nakazawa et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:360-364), the latter of which can be particularly useful for detecting point mutations in the SARP3 gene (see Abravaya et al. (1995) *Nucleic Acids Res* 0.23:675-682). This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (e.g., genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a SARP3 gene under conditions such that hybridization and amplification of the SARP3 gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. PCR and/or LCR sensitivity can be enhanced by use of a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

[0083] Alternative amplification methods include: self sustained sequence replication (Guatelli, J. C. et al., (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, D. Y. et al., (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P. M. et al. (1988) *Bio-Technology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

[0084] In an alternative embodiment, mutations in a SARP3 gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA are isolated, optionally amplified, then digested with one or more restriction endonucleases. Fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicate mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Pat. No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

[0085] In other embodiments, genetic mutations in SARP3 can be identified by hybridizing a sample and control nucleic acids, e.g., DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin, M. T. et al. (1996) *Human Mutation* 7: 244-255; Kozal, M. J. et al. (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in SARP3 can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M. T. et al. supra. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

[0086] In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the SARP3 gene and detect mutations by comparing the sequence of the sample SARP3 with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxam and Gilbert ((1977) *Proc. Natl. Acad. Sci. USA* 74:560) or Sanger ((1977) *Proc. Natl. Acad. Sci. USA* 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry (see, e.g., PCT Publication No. WO 94/16101; Cohen et al. (1996) *Adv. Chromatogr.* 36:127-162; and Griffin et al. (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

[0087] Other methods for detecting mutations in the SARP3 gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers et al. (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing

the wild-type SARP3 sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digest the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton et al. (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba et al. (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

[0088] In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in SARP3 cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu et al. (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a SARP3 sequence, e.g., a wild-type SARP3 sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Pat. No. 5,459,039.

[0089] In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in SARP3 genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita et al. (1989) *Proc Natl. Acad. Sci USA:* 86:2766, see also Cotton (1993) *Mutat. Res.* 285:125-144; and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control SARP3 nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen et al. (1991) *Trends Genet* 7:5).

[0090] In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers et al. (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example, by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

[0091] Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki et al. (1986) *Nature* 324:163); Saiki et al. (1989) *Proc. Natl Acad. Sci USA* 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

[0092] Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs et al. (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) Tibtech 11:238). In addition, it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini et al. (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

[0093] The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family history of a metabolic disease or illness involving a SARP3 gene.

[0094] Furthermore, any cell type or tissue in which SARP3 is expressed may be utilized in the prognostic assays described herein.

[0095] C. Monitoring of Effects During Clinical Trials

[0096] Monitoring the influence of agents (e.g., drugs) on the expression or activity of a SARP3 polypeptide (e.g., the modulation of an enzymatic or catalytic activity) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase SARP3 gene expression, polypeptide levels, or upregulate SARP3 activity, can be monitored in clinical trials of subjects exhibiting decreased SARP3 gene expression, polypeptide levels, or downregulated SARP3 activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease SARP3 gene expression, polypeptide levels, or downregulate SARP3 activity, can be monitored in clinical

trials of subjects exhibiting increased SARP3 gene expression, polypeptide levels, or upregulated SARP3 activity. In such clinical trials, the expression or activity of a SARP3 gene, and preferably, other genes that have been implicated in, for example, a SARP3-associated disorder, e.g., a metabolic disease or disorder, can be used as a "read out" or markers of the phenotype of a particular cell.

[0097] For example, and not by way of limitation, genes, including SARP3, that are modulated in cells by treatment with an agent (e.g., compound, drug or small molecule) which modulates SARP3 activity (e.g., identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on metabolic disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of SARP3 and other genes implicated in the metabolic disorder, respectively. The levels of gene expression (e.g., a gene expression pattern) can be quantified by northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of polypeptide produced, by one of the methods as described herein, or by measuring the levels or activity of SARP3 or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during treatment of the individual with the agent.

[0098] In a preferred embodiment, the invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) including the steps of: (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a SARP3 polypeptide, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the SARP3 polypeptide, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the SARP3 polypeptide, mRNA, or genomic DNA in the pre-administration sample with the SARP3 polypeptide, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of SARP3 to higher levels than detected, i.e., to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of SARP3 to lower levels than detected, i.e. to decrease the effectiveness of the agent. According to such an embodiment, SARP3 expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

[0099] D. Electronic Apparatus Readable Media and Arrays

[0100] Electronic apparatus readable media comprising SARP3 sequence information is also provided. As used herein, "SARP3 sequence information" refers to any nucleotide and/or amino acid sequence information particular to the SARP3 molecules of the invention, including but not limited to full-length nucleotide and/or amino acid sequences, partial nucleotide and/or amino acid sequences, polymorphic sequences including single nucleotide polymorphisms (SNPs), epitope sequences, and the like. Moreover, information "related to" said SARP3 sequence information includes detection of the presence or absence of a sequence (e.g., detection of expression of a sequence, fragment, polymorphism, etc.), determination of the level of a sequence (e.g., detection of a level of expression, for example, a quantitative detection), detection of a reactivity to a sequence (e.g., detection of protein expression and/or levels, for example, using a sequence-specific antibody), and the like. As used herein, "electronic apparatus readable media" refers to any suitable medium for storing, holding or containing data or information that can be read and accessed directly by an electronic apparatus. Such media can include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as compact disc; electronic storage media such as RAM, ROM, EPROM, EEPROM and the like; general hard disks and hybrids of these categories such as magnetic/optical storage media. The medium is adapted or configured for having recorded thereon SARP3 sequence information of the invention.

[0101] As used herein, the term "electronic apparatus" is intended to include any suitable computing or processing apparatus or other device configured or adapted for storing data or information. Examples of electronic apparatus suitable for use with the invention include stand-alone computing apparatus; networks, including a local area network (LAN), a wide area network (WAN) Internet, Intranet, and Extranet; electronic appliances such as a personal digital assistants (PDAs), cellular phone, pager and the like; and local and distributed processing systems.

[0102] As used herein, "recorded" refers to a process for storing or encoding information on the electronic apparatus readable medium. Those skilled in the art can readily adopt any of the presently known methods for recording information on known media to generate manufactures comprising the SARP3 sequence information.

[0103] A variety of software programs and formats can be used to store the sequence information on the electronic apparatus readable medium. For example, the sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and MicroSoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like, as well as in other forms. Any number of dataprocessor structuring formats (e.g., text file or database) may be employed in order to obtain or create a medium having recorded thereon the SARP3 sequence information.

[0104] By providing SARP3 sequence information in readable form, one can routinely access the sequence information for a variety of purposes. For example, one skilled in the art can use the sequence information in readable form to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments or regions of the sequences of the invention which match a particular target sequence or target motif.

[0105] The invention therefore provides a medium for holding instructions for performing a method for determin-

ing whether a subject has a SARP3-associated, e.g., a metabolic, disease or disorder or a pre-disposition to a SARP3-associated, e.g., a metabolic, disease or disorder, wherein the method comprises the steps of determining SARP3 sequence information associated with the subject and based on the SARP3 sequence information, determining whether the subject has a SARP3-associated disease or disorder or a pre-disposition to a SARP3-associated disease or disorder and/or recommending a particular treatment for the disease, disorder or pre-disease condition.

[0106] The invention further provides in an electronic system and/or in a network, a method for determining whether a subject has a SARP3-associated, e.g., a metabolic, disease or disorder or a pre-disposition to a disease associated with a SARP3, wherein the method comprises the steps of determining SARP3 sequence information associated with the subject, and based on the SARP3 sequence information, determining whether the subject has a SARP3-associated disease or disorder or a pre-disposition to a SARP3-associated disease or disorder, and/or recommending a particular treatment for the disease, disorder or pre-disease condition. The method may further comprise the step of receiving phenotypic information associated with the subject and/or acquiring from a network phenotypic information associated with the subject.

[0107] The invention also provides in a network, a method for determining whether a subject has a SARP3-associated, e.g., a metabolic, disease or disorder or a pre-disposition to a SARP3-associated disease or disorder associated with SARP3, said method comprising the steps of receiving SARP3 sequence information from the subject and/or information related thereto, receiving phenotypic information associated with the subject, acquiring information from the network corresponding to SARP3 and/or a SARP3-associated disease or disorder, and based on one or more of the phenotypic information, the SARP3 information (e.g., sequence information and/or information related thereto), and the acquired information, determining whether the subject has a SARP3-associated disease or disorder or a pre-disposition to a SARP3-associated disease or disorder. The method may further comprise the step of recommending a particular treatment for the disease, disorder or pre-disease condition.

[0108] The invention also provides a business method for determining whether a subject has a SARP3-associated, e.g., a metabolic, disease or disorder or a pre-disposition to a SARP3-associated disease or disorder, said method comprising the steps of receiving information related to SARP3 (e.g., sequence information and/or information related thereto), receiving phenotypic information associated with the subject, acquiring information from the network related to SARP3 and/or related to a SARP3-associated disease or disorder, and based on one or more of the phenotypic information, the SARP3 information, and the acquired information, determining whether the subject has a SARP3-associated disease or disorder or a pre-disposition to a SARP3-associated disease or disorder. The method may further comprise the step of recommending a particular treatment for the disease, disorder or pre-disease condition.

[0109] The invention also includes an array comprising a SARP3 sequence of the invention. The array can be used to assay expression of one or more genes in the array. In one embodiment, the array can be used to assay gene expression in a tissue to ascertain tissue specificity of genes in the array. In this manner, up to about 7600 genes can be simultaneously assayed for expression, one of which can be SARP3. This allows a profile to be developed showing a battery of genes specifically expressed in one or more tissues.

[0110] In addition to such qualitative determination, the invention allows the quantitation of gene expression. Thus, not only tissue specificity, but also the level of expression of a battery of genes in the tissue is ascertainable. Thus, genes can be grouped on the basis of their tissue expression per se and level of expression in that tissue. This is useful, for example, in ascertaining the relationship of gene expression between or among tissues. Thus, one tissue can be perturbed and the effect on gene expression in a second tissue can be determined. In this context, the effect of one cell type on another cell type in response to a biological stimulus can be determined. Such a determination is useful, for example, for determining the effect of cell-cell interactions at the level of gene expression. If an agent is administered therapeutically to treat one cell type but has an undesirable effect on another cell type, the invention provides an assay to determine the molecular basis of the undesirable effect and thus provides the opportunity to co-administer a counteracting agent or otherwise treat the undesired effect. Similarly, even within a single cell type, undesirable biological effects can be determined at the molecular level. Thus, the effects of an agent on expression of other than the target gene can be ascertained and counteracted.

[0111] In another embodiment, the array can be used to monitor the time course of expression of one or more genes in the array. This can occur in various biological contexts, as disclosed herein, for example development of a SARP3-associated, e.g., a metabolic, disease or disorder, progression of SARP3-associated disease or disorder, and processes, such a cellular transformation associated with the SARP3-associated disease or disorder.

[0112] The array is also useful for ascertaining the effect of the expression of a gene on the expression of other genes in the same cell or in different cells (e.g., ascertaining the effect of SARP3 expression on the expression of other genes). This provides, for example, for a selection of alternate molecular targets for therapeutic intervention if the ultimate or downstream target cannot be regulated.

[0113] The array is also useful for ascertaining differential expression patterns of one or more genes in normal and abnormal cells. This provides a battery of genes (e.g., including SARP3) that could serve as a molecular target for diagnosis or therapeutic intervention.

[0114] III. Methods of Treatment of Subjects Suffering from Metabolic Disorders:

[0115] The invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant or unwanted SARP3 expression or activity, e.g. a metabolic disorder such as obesity or diabetes. With regard to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein,

refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of how a patient's genes determine his or her response to a drug (e.g., a patient's "drug response phenotype", or "drug response genotype"). Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the SARP3 molecules of the invention or SARP3 modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

[0116] Treatment is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease or the predisposition toward disease.

[0117] A therapeutic agent includes, but is not limited to, small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides.

[0118] A. Prophylactic Methods

[0119] In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant or unwanted SARP3 expression or activity, by administering to the subject a SARP3 or an agent which modulates SARP3 expression or at least one SARP3 activity. Subjects at risk for a disease which is caused or contributed to by aberrant or unwanted SARP3 expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the SARP3 aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of SARP3 aberrancy, for example, a SARP3 molecule, SARP3 agonist or SARP3 antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

[0120] B. Therapeutic Methods

[0121] The SARP3 nucleic acid molecules, fragments of SARP3 polypeptides, and anti-SARP3 antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, polypeptide, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[0122] A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0123] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[0124] Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a fragment of a SARP3 polypeptide or an anti-SARP3 antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0125] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[0126] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

[0127] For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[0128] The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[0129] In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation (Palo Alto, Calif.) and Alkermes (Cambridge Mass.). Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

[0130] It is especially advantageous to formulate oral or parenteral compositions in "dosage unit form" for ease of administration and uniformity of dosage. "Dosage unit form", as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

[0131] Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[0132] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

[0133] As defined herein, a therapeutically effective amount of polypeptide (i.e., an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a polypeptide or antibody can include a single treatment or, preferably, can include a series of treatments.

[0134] In a preferred example, a subject is treated with antibody or polypeptide in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

[0135] The invention encompasses agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (i.e., including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds. It is understood that appropriate doses of small molecule agents depends upon a number of factors within the ken of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention.

[0136] Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (e.g., about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays described herein. When one or more of these small molecules is to be administered to an animal (e.g., a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

[0137] Further, an antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologues thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

[0138] The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

[0139] Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982). Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Pat. No. 4,676,980.

[0140] The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Pat. No. 5,328,470) or by stereotactic injection (see e.g., Chen et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

[0141] The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

[0142] C. Pharmacogenomics

[0143] The SARP3 molecules of the invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on SARP3 activity (e.g., SARP3 gene expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) metabolic disorders (e.g., proliferative disorders) associated with aberrant or unwanted SARP3 activity. In conjunction with such treatment, pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a SARP3 molecule or SARP3 modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with a SARP3 molecule or SARP3 modulator.

[0144] Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. et al. (1996) *Clin. Exp. Pharmacol. Physiol.* 23(10-11): 983-985 and Linder, M. W. et al. (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

[0145] One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (e.g., a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

[0146] Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drugs target is known (e.g., a SARP3 polypeptide of the invention), all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

[0147] As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (e.g., N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

[0148] Alternatively, a method termed the "gene expression profiling", can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (e.g., a SARP3 molecule or SARP3 modulator of the invention) can give an indication whether gene pathways related to toxicity have been turned on.

[0149] Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a SARP3 molecule or SARP3 modulator, such as a modulator identified by one of the exemplary screening assays described herein.

[0150] IV. Recombinant Expression Vectors and Host Cells Used in the Methods of the Invention

[0151] The methods of the invention (e.g., the screening assays described herein) include the use of vectors, preferably expression vectors, containing a nucleic acid encoding a SARP3 protein, or a portion thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked.

One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

[0152] The recombinant expression vectors to be used in the methods of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an in vitro transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel (1990) *Methods Enzymol.* 185:3-7. Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cells and those which direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., SARP3 proteins, mutant forms of SARP3 proteins, fusion proteins, and the like).

[0153] The recombinant expression vectors to be used in the methods of the invention can be designed for expression of SARP3 proteins in prokaryotic or eukaryotic cells. For example, SARP3 proteins can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors), yeast cells, or mammalian cells. Suitable host cells are discussed further in Goeddel (1990) supra. Alternatively, the recombinant expression vector can be transcribed and translated in vitro, for example using T7 promoter regulatory sequences and T7 polymerase.

[0154] Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D. B. and Johnson, K. S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

[0155] Purified fusion proteins can be utilized in SARP3 activity assays, (e.g., direct assays or competitive assays described in detail below), or to generate antibodies specific for SARP3 proteins. In a preferred embodiment, a SARP3 fusion protein expressed in a retroviral expression vector of the invention can be utilized to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (e.g., six weeks).

[0156] In another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman et al. (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J. et al., *Molecular Cloning: A Laboratory Manual.* 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

[0157] In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid).

[0158] The methods of the invention may further use a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to SARP3 mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the anti-

sense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific, or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid, or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes, see Weintraub, H. et al., Antisense RNA as a molecular tool for genetic analysis, *Reviews—Trends in Genetics, Vol.* 1(1) 1986.

[0159] Another aspect of the invention pertains to the use of host cells into which a SARP3 nucleic acid molecule of the invention is introduced, e.g., a SARP3 nucleic acid molecule within a recombinant expression vector or a SARP3 nucleic acid molecule containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

[0160] A host cell can be any prokaryotic or eukaryotic cell. For example, a SARP3 protein can be expressed in bacterial cells such as *E. coli,* insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

[0161] Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook et al. (*Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals.

[0162] A host cell used in the methods of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (i.e., express) a SARP3 protein. Accordingly, the invention further provides methods for producing a SARP3 protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of the invention (into which a recombinant expression vector encoding a SARP3 protein has been introduced) in a suitable medium such that a SARP3 protein is produced. In another embodiment, the method further comprises isolating a SARP3 protein from the medium or the host cell.

[0163] V. Isolated Nucleic Acid Molecules used in the Methods of the Invention

[0164] The coding sequence of the isolated human SARP3, also referred to herein as SFRP5 or 17537, cDNA and the predicted amino acid sequence of the human SARP3 polypeptide are shown in SEQ ID NOs: 1 and 2, respectively. The sequence of the coding region in SEQ ID NO:1, residues 13 to 1425, is shown in SEQ ID NO:3. The SARP3 polynucleotide and amino acid sequences are identical with sequences in GenBank Accession No. NM_003015, and are also described in PCT Publication No. WO 98/13493, the contents of which are incorporated herein by reference.

[0165] The coding sequence of the isolated mouse SARP3, also referred to herein as 17540, cDNA and the predicted amino acid sequence of the mouse SARP3 polypeptide are shown in SEQ ID NOs:4 and 5, respectively. The sequence of the coding region in SEQ ID NO:4 is shown in SEQ ID NO:6. The mouse SARP3 polynucleotide and amino acid sequences are identical with sequences in GenBank Accession No. AF117759.

[0166] The methods of the invention include the use of isolated nucleic acid molecules that encode SARP3 proteins, or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify SARP3 encoding nucleic acid molecules (e.g., SARP3 mRNA) and fragments for use as PCR primers for the amplification or mutation of SARP3 nucleic acid molecules. As used herein, the term "*nucleic acid molecule*" is intended to include DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

[0167] A nucleic acid molecule used in the methods of the invention, e.g., a nucleic acid molecule having the nucleotide sequence of SEQ ID NO: 1, 3, 4, or 6, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or portion of the nucleic acid sequence of SEQ ID NO: 1, 3, 4, or 6 as a hybridization probe, SARP3 nucleic acid molecules can be isolated using standard hybridization and cloning techniques (e.g., as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual.* 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989).

[0168] Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO: 1, 3, 4, or 6 can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO: 1, 3, 4, or 6.

[0169] A nucleic acid used in the methods of the invention can be amplified using cDNA, mRNA or, alternatively, genomic DNA as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. Furthermore, oligonucleotides corresponding to SARP3 nucleotide sequences can be prepared by standard synthetic techniques, e.g., using an automated DNA synthesizer.

[0170] In a preferred embodiment, the isolated nucleic acid molecules used in the methods of the invention comprise the nucleotide sequence shown in SEQ ID NO: 1, 3, 4, or 6, a complement of the nucleotide sequence shown in SEQ ID NO: 1, 3, 4, or 6, or a portion of any of these nucleotide sequences. A nucleic acid molecule which is

complementary to the nucleotide sequence shown in SEQ ID NO: 1, 3, 4, or 6, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6 such that it can hybridize to the nucleotide sequence shown in SEQ ID NO: 1, 3, 4, or 6 thereby forming a stable duplex.

[0171] In still another preferred embodiment, an isolated nucleic acid molecule used in the methods of the invention comprises a nucleotide sequence which is at least about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the entire length of the nucleotide sequence shown in SEQ ID NO: 1, 3, 4, or 6, or a portion of any of this nucleotide sequence.

[0172] Moreover, the nucleic acid molecules used in the methods of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO: 1, 3, 4, or 6, for example, a fragment which can be used as a probe or primer or a fragment encoding a portion of a SARP3 protein, e.g., a biologically active portion of a SARP3 protein. The probe or primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a sense sequence of SEQ ID NO: 1, 3, 4, or 6 of an anti-sense sequence of SEQ ID NO: 1, 3, 4, or 6, or of a naturally occurring allelic variant or mutant of SEQ ID NO: 1, 3, 4, or 6. In one embodiment, a nucleic acid molecule used in the methods of the invention comprises a nucleotide sequence which is greater than 100, 100-200, 200-300, 300-400, 400-500, 500-600, or more nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO: 1, 3, 4, or 6.

[0173] As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences that are significantly identical or homologous to each other remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% identical to each other remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, Ausubel et al., eds., John Wiley & Sons, Inc. (1995), sections 2, 4 and 6. Additional stringent conditions can be found in *Molecular Cloning: A Laboratory Manual*, Sambrook et al., Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989), chapters 7, 9 and 11. A preferred, non-limiting example of stringent hybridization conditions includes hybridization in 4×sodium chloride/sodium citrate (SSC), at about 65-70° C. (or hybridization in 4×SSC plus 50% formamide at about 42-50° C.) followed by one or more washes in 1×SSC, at about 65-70° C. A preferred, non-limiting example of highly stringent hybridization conditions includes hybridization in 1×SSC, at about 65-70° C. (or hybridization in 1×SSC plus 50% formamide at about 42-50° C.) followed by one or more washes in 0.3×SSC, at about 65-70° C. A preferred, non-limiting example of reduced stringency hybridization conditions includes hybridization in 4×SSC, at about 50-60° C. (or alternatively hybridization in 6×SSC plus 50% formamide at about 40-45° C.) followed by one or more washes in 2×SSC, at about 50-60° C. Ranges intermediate to the above-recited values, e.g., at 65-70° C. or at 42-50° C. are also intended to be encompassed by the invention. SSPE (1×SSPE is 0.15M NaCl, 10 mM NaH_2PO_4, and 1.25 mM EDTA, pH 7.4) can be substituted for SSC (1×SSC is 0.15M NaCl and 15 mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes each after hybridization is complete. The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10° C. less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, T_m(° C.)=2(# of A+T bases)+4(# of G+C bases). For hybrids between 18 and 49 base pairs in length, T_m(° C.)=81.5+16.6($\log_{10}[Na^+]$)+0.41(% G+C)−(600/N), where N is the number of bases in the hybrid, and $[Na^+]$ is the concentration of sodium ions in the hybridization buffer ($[Na^+]$ for 1×SSC=0.165 M). It will also be recognized by the skilled practitioner that additional reagents may be added to hybridization and/or wash buffers to decrease non-specific hybridization of nucleic acid molecules to membranes, for example, nitrocellulose or nylon membranes, including but not limited to blocking agents (e.g., BSA or salmon or herring sperm carrier DNA), detergents (e.g., SDS), chelating agents (e.g., EDTA), Ficoll, PVP and the like. When using nylon membranes, in particular, an additional preferred, non-limiting example of stringent hybridization conditions is hybridization in 0.25-0.5M NaH_2PO_4, 7% SDS at about 65° C., followed by one or more washes at 0.02M NaH_2PO_4, 1% SDS at 65° C., see e.g., Church and Gilbert (1984) *Proc. Natl. Acad. Sci. USA* 81:1991-1995, (or alternatively 0.2×SSC, 1% SDS).

[0174] In preferred embodiments, the probe further comprises a label group attached thereto, e.g., the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a SARP3 protein, such as by measuring a level of a SARP3-encoding nucleic acid in a sample of cells from a subject e.g., detecting SARP3 mRNA levels or determining whether a genomic SARP3 gene has been mutated or deleted.

[0175] The methods of the invention further encompass the use of nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO: 1, 3, 4, or 6 due to degeneracy of the genetic code and thus encode the same SARP3 proteins as those encoded by the nucleotide sequence shown in SEQ ID NO: 1, 3, 4, or 6. In another embodiment, an isolated nucleic acid molecule included in the methods of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2 or 5.

[0176] The methods of the invention further include the use of allelic variants of human and/or mouse SARP3, e.g., functional and non-functional allelic variants. Functional allelic variants are naturally occurring amino acid sequence variants of the human and/or mouse SARP3 protein that maintain a SARP3 activity. Functional allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2 or 5, or substitution, deletion or insertion of non-critical residues in non-critical regions of the protein.

[0177] Non-functional allelic variants are naturally occurring amino acid sequence variants of the human and/or mouse SARP3 protein that do not have a SARP3 activity. Non-functional allelic variants will typically contain a non-conservative substitution, deletion, or insertion or premature truncation of the amino acid sequence of SEQ ID NO:2 or 5, or a substitution, insertion or deletion in critical residues or critical regions of the protein.

[0178] The methods of the invention may further use non-human orthologues of the human and/or mouse SARP3 protein. Orthologues of the human and/or mouse SARP3 protein are proteins that are isolated from non-human organisms and possess the same SARP3 activity.

[0179] The methods of the invention further include the use of nucleic acid molecules comprising the nucleotide sequence of SEQ ID NO: 1, 3, 4, or 6, or a portion thereof, in which a mutation has been introduced. The mutation may lead to amino acid substitutions at "non-essential" amino acid residues or at "essential" amino acid residues. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of SARP3 (e.g., the sequence of SEQ ID NO:2 or 5) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the SARP3 proteins of the invention are not likely to be amenable to alteration.

[0180] Mutations can be introduced into SEQ ID NO: 1, 3, 4, or 6 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a SARP3 protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a SARP3 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for SARP3 biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO: 1, 3, 4, or 6, the encoded protein can be expressed recombinantly and the activity of the protein can be determined using the assay described herein.

[0181] Another aspect of the invention pertains to the use of isolated nucleic acid molecules which are antisense to the nucleotide sequence of SEQ ID NO: 1, 3, 4, or 6. An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire SARP3 coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding a SARP3. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding SARP3. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (also referred to as 5' and 3' untranslated regions).

[0182] Given the coding strand sequences encoding SARP3 disclosed herein, antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of SARP3 mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of SARP3 mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of SARP3 mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine,
5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

[0183] The antisense nucleic acid molecules used in the methods of the invention are typically administered to a subject or generated in situ such that they hybridize with or

bind to cellular mRNA and/or genomic DNA encoding a SARP3 protein to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

[0184] In yet another embodiment, the antisense nucleic acid molecule used in the methods of the invention is an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gaultier et al. (1987) *Nucleic Acids. Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue et al. (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue et al. (1987) *FEBS Lett.* 215:327-330).

[0185] In still another embodiment, an antisense nucleic acid used in the methods of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave SARP3 mRNA transcripts to thereby inhibit translation of SARP3 mRNA. A ribozyme having specificity for a SARP3-encoding nucleic acid can be designed based upon the nucleotide sequence of a SARP3 cDNA disclosed herein (i.e., SEQ ID NO: 1, 3, 4, or 6). For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a SARP3-encoding mRNA. See, e.g., U.S. Pat. Nos. 4,987,071 and 5,116,742. Alternatively, SARP3 mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel, D. and Szostak, J. W. (1993) *Science* 261:1411-1418.

[0186] Alternatively, SARP3 gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the SARP3 (e.g., the SARP3 promoter and/or enhancers) to form triple helical structures that prevent transcription of the SARP3 gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6): 569-84; Helene, C. et al. (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L. J. (1992) *Bioassays* 14(12):807-15.

[0187] In yet another embodiment, the SARP3 nucleic acid molecules used in the methods of the invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. et al. (1996) *Bioorganic & Medicinal Chemistry* 4:5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, e.g., DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. et al. (1996) supra; Perry-O'Keefe et al. (1996) *Proc. Natl. Acad. Sci.* 93:14670-675.

[0188] PNAs of SARP3 nucleic acid molecules can be used in the therapeutic and diagnostic applications described herein. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of SARP3 nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (e.g., by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (e.g., S1 nucleases (Hyrup B. et al. (1996) supra)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. et al. (1996) supra; Perry-O'Keefe et al. (1996) supra).

[0189] In another embodiment, PNAs of SARP3 can be modified, (e.g., to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of SARP3 nucleic acid molecules can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, (e.g., RNAse H and DNA polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup B. et al. (1996) supra). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup B. et al. (1996) supra and Finn P. J. et al. (1996) *Nucleic Acids Res.* 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs, e.g., 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. et al. (1989) *Nucleic Acid Res.* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P. J. et al. (1996) supra). Alternatively, chimeric molecules can be synthesized with a 5' DNA

segment and a 3'PNA segment (Peterser, K. H. et al. (1975) *Bioorganic Med. Chem. Lett.* 5: 1119-11124.

[0190] In other embodiments, the oligonucleotide used in the methods of the invention may include other appended groups such as peptides (e.g., for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. WO88/09810) or the blood-brain barrier (see, e.g., PCT Publication No. WO89/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, e.g., Krol et al. (1988) *Bio-Techniques* 6:958-976) or intercalating agents. (See, e.g., Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

[0191] VI. Isolated SARP3 Proteins and Anti-SARP3 Antibodies used in the Methods of the Invention

[0192] The methods of the invention include the use of isolated SARP3 proteins, and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to raise anti-SARP3 antibodies. In one embodiment, native SARP3 proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, SARP3 proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a SARP3 protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

[0193] As used herein, a "biologically active portion" of a SARP3 protein includes a fragment of a SARP3 protein having a SARP3 activity. Biologically active portions of a SARP3 protein include peptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of the SARP3 protein, e.g., the amino acid sequence shown in SEQ ID NO:2 or 5, which include fewer amino acids than the full length SARP3 proteins, and exhibit at least one activity of a SARP3 protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the SARP3 protein (e.g., the N-terminal region of the SARP3 protein that is believed to be involved in the regulation of apoptotic activity). A biologically active portion of a SARP3 protein can be a polypeptide which is, for example, 25, 50, 75, 100, 125, 150, 175, 200, 250, 300 or more amino acids in length. Biologically active portions of a SARP3 protein can be used as targets for developing agents which modulate a SARP3 activity.

[0194] In a preferred embodiment, the SARP3 protein used in the methods of the invention has an amino acid sequence shown in SEQ ID NO:2 or 5. In other embodiments, the SARP3 protein is substantially identical to SEQ ID NO:2 or 5, and retains the functional activity of the protein of SEQ ID NO:2 or 5, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail in subsection V above. Accordingly, in another embodiment, the SARP3 protein used in the methods of the invention is a protein which comprises an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:2 or 5.

[0195] To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence (e.g., when aligning a second sequence to the SARP3 amino acid sequence of SEQ ID NO:2 or 5 having 361 amino acid residues, at least 94, preferably at least 126, more preferably at least 158, more preferably at least 189, even more preferably at least 221, and even more preferably at least 252 or 284 or more amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

[0196] The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blosum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at http://www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (*Comput. Appl. Biosci.* 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0 or 2.0U), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

[0197] The methods of the invention may also use SARP3 chimeric or fusion proteins. As used herein, a SARP3 "chimeric protein" or "fusion protein" comprises a SARP3 polypeptide operatively linked to a non-SARP3 polypeptide. An "SARP3 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a SARP3 molecule, whereas a "non-SARP3 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the SARP3 protein, e.g., a protein which is different from the SARP3 protein and which is derived from the same or a different organism. Within a SARP3 fusion protein the SARP3

polypeptide can correspond to all or a portion of a SARP3 protein. In a preferred embodiment, a SARP3 fusion protein comprises at least one biologically active portion of a SARP3 protein. In another preferred embodiment, a SARP3 fusion protein comprises at least two biologically active portions of a SARP3 protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the SARP3 polypeptide and the non-SARP3 polypeptide are fused in-frame to each other. The non-SARP3 polypeptide can be fused to the N-terminus or C-terminus of the SARP3 polypeptide.

[0198] For example, in one embodiment, the fusion protein is a GST-SARP3 fusion protein in which the SARP3 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant SARP3.

[0199] In another embodiment, this fusion protein is a SARP3 protein containing a heterologous signal sequence at its N-terminus. In certain host cells (e.g., mammalian host cells), expression and/or secretion of SARP3 can be increased through use of a heterologous signal sequence.

[0200] The SARP3 fusion proteins used in the methods of the invention can be incorporated into pharmaceutical compositions and administered to a subject in vivo. The SARP3 fusion proteins can be used to affect the bioavailability of a SARP3 substrate. Use of SARP3 fusion proteins may be useful therapeutically for the treatment of disorders caused by, for example, (i) aberrant modification or mutation of a gene encoding a SARP3 protein; (ii) mis-regulation of the SARP3 gene; and (iii) aberrant post-translational modification of a SARP3 protein.

[0201] Moreover, the SARP3-fusion proteins used in the methods of the invention can be used as immunogens to produce anti-SARP3 antibodies in a subject, to purify SARP3 ligands and in screening assays to identify molecules which inhibit the interaction of SARP3 with a SARP3 substrate.

[0202] Preferably, a SARP3 chimeric or fusion protein used in the methods of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel et al. John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). A SARP3-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the SARP3 protein.

[0203] The invention also pertains to the use of variants of the SARP3 proteins which function as either SARP3 agonists (mimetics) or as SARP3 antagonists. Variants of the SARP3 proteins can be generated by mutagenesis, e.g., discrete point mutation or truncation of a SARP3 protein. An agonist of the SARP3 proteins can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of a SARP3 protein. An antagonist of a SARP3 protein can inhibit one or more of the activities of the naturally occurring form of the SARP3 protein by, for example, competitively modulating a SARP3-mediated activity of a SARP3 protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the SARP3 protein.

[0204] In one embodiment, variants of a SARP3 protein which function as either SARP3 agonists (mimetics) or as SARP3 antagonists can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of a SARP3 protein for SARP3 protein agonist or antagonist activity. In one embodiment, a variegated library of SARP3 variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of SARP3 variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential SARP3 sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display) containing the set of SARP3 sequences therein. There are a variety of methods which can be used to produce libraries of potential SARP3 variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential SARP3 sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, e.g., Narang, S. A. (1983) *Tetrahedron* 39:3; Itakura et al. (1984) *Annu. Rev. Biochem.* 53:323; Itakura et al. (1984) *Science* 198:1056; Ike et al. (1983) *Nucleic Acid Res.* 11:477).

[0205] In addition, libraries of fragments of a SARP3 protein coding sequence can be used to generate a variegated population of SARP3 fragments for screening and subsequent selection of variants of a SARP3 protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a SARP3 coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the SARP3 protein.

[0206] Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries

for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of SARP3 proteins. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify SARP3 variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave et al. (1993) *Protein Engineering* 6(3):327-331).

[0207] The methods of the invention further include the use of anti-SARP3 antibodies. An isolated SARP3 protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind SARP3 using standard techniques for polyclonal and monoclonal antibody preparation. A full-length SARP3 protein can be used or, alternatively, antigenic peptide fragments of SARP3 can be used as immunogens. The antigenic peptide of SARP3 comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2 or 5 and encompasses an epitope of SARP3 such that an antibody raised against the peptide forms a specific immune complex with the SARP3 protein. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

[0208] Preferred epitopes encompassed by the antigenic peptide are regions of SARP3 that are located on the surface of the protein, e.g., hydrophilic regions, as well as regions with high antigenicity.

[0209] A SARP3 immunogen is typically used to prepare antibodies by immunizing a suitable subject, (e.g., rabbit, goat, mouse, or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed SARP3 protein or a chemically synthesized SARP3 polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic SARP3 preparation induces a polyclonal anti-SARP3 antibody response.

[0210] The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as a SARP3. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and $F(ab)_2$ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind SARP3 molecules. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of SARP3. A monoclonal antibody composition thus typically displays a single binding affinity for a particular SARP3 protein with which it immunoreacts.

[0211] Polyclonal anti-SARP3 antibodies can be prepared as described above by immunizing a suitable subject with a SARP3 immunogen. The anti-SARP3 antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized SARP3. If desired, the antibody molecules directed against SARP3 can be isolated from the mammal (e.g., from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, e.g., when the anti-SARP3 antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown et al. (1981) *J. Immunol.* 127:539-46; Brown et al. (1980) *J. Biol. Chem.* 255:4980-83; Yeh et al. (1976) *Proc. Natl. Acad. Sci. USA* 76:2927-31; and Yeh et al. (1982) *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor et al. (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole et al. (1985) *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally Kenneth, R. H. in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, N.Y. (1980); Lerner, E. A. (1981) *Yale J. Biol. Med.* 54:387-402; Gefter, M. L. et al. (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a SARP3 immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds SARP3.

[0212] Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-SARP3 monoclonal antibody (see, e.g., G. Galfre et al. (1977) *Nature* 266:55052; Gefter et al. (1977) supra; Lerner (1981) supra; and Kenneth (1980) supra). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (e.g., a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, e.g., the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days

because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind SARP3, e.g., using a standard ELISA assay.

[0213] Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-SARP3 antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with SARP3 to thereby isolate immunoglobulin library members that bind SARP3. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit, Catalog No.* 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, U.S. Pat. No. 5,223,409; PCT Publication No. WO 92/18619; PCT Publication No. WO 91/17271; PCT Publication No. WO 92/20791; PCT Publication No. WO 92/15679; PCT Publication No. WO 93/01288; PCT Publication No. WO 92/01047; PCT Publication No. WO 92/09690; PCT Publication No. WO 90/02809; Fuchs et al. (1991) *Bio/Technology* 9:1370-1372; Hay et al. (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse et al. (1989) *Science* 246:1275-1281; Griffiths et al. (1993) *EMBO J* 12:725-734; Hawkins et al. (1992) *J. Mol. Biol.* 226:889-896; Clarkson et al. (1991) *Nature* 352:624-628; Gram et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:3576-3580; Garrad et al. (1991) *Bio/Technology* 9:1373-1377; Hoogenboom et al. (1991) *Nuc. Acid Res.* 19:4133-4137; Barbas et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:7978-7982; and McCafferty et al. (1990) *Nature* 348:552-554.

[0214] Additionally, recombinant anti-SARP3 antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the methods of the *invention*. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Kobinson et al. International Application No. PCT/US86/02269; Akira, et al. European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison et al. European Patent Application 173,494; Neuberger et al. PCT Publication No. WO 86/01533; U.S. Pat. No. 4,816,567; Cabilly et al. European Patent 125,023; Better et al. (1988) *Science* 240:1041-1043; Liu et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu et al. (1987) *J. Immunol.* 139:3521-3526; Sun et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura et al. (1987) *Canc. Res.* 47:999-1005; Wood et al. (1985) *Nature* 314:446-449; Shaw et al. (1988) *J. Natl. Cancer Inst.* 80:1553-1559; Morrison, S. L. (1985) *Science* 229:1202-1207; Oi et al. (1986) *BioTechniques* 4:214; U.S. Pat. No. 5,225,539; Jones et al. (1986) *Nature* 321:552-525; Verhoeyan et al. (1988) *Science* 239:1534; and Beidler et al. (1988) *J. Immunol.* 141:4053-4060.

[0215] An anti-SARP3 antibody can be used to detect SARP3 protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the SARP3 protein. Anti-SARP3 antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, □-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

[0216] This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Sequence Listing is incorporated herein by reference.

EXAMPLES

Example 1

SARP3 Gene Expression in Human Tissues

[0217] Northern blot analysis was used to evaluate expression of SARP3 in human tissues. The results indicated a high level of expression in pancreas, followed by a lower level in spinal cord, lower levels in liver and small intestine, and a detectable level in colon.

[0218] SARP3 expression was also analyzed in human tissue samples collected from abdominal subcutaneous fat and adipose tissue from the buttocks of ten nonobese adult men and women, which included five volunteers subjected to a high calorie, fat-rich diet over an eight-week period.

[0219] Total RNA was prepared using the trizol method and treated with DNase to remove contaminating genomic DNA. cDNA was synthesized using standard techniques. Mock cDNA synthesis in the absence of reverse transcriptase resulted in samples with no detectable PCR amplification of the control 18S gene, confirming efficient removal of genomic DNA contamination. SARP3 expression was measured by TaqMan quantitative PCR analysis, performed according to the manufacturer's directions (Perkin Elmer Applied Biosystems, Foster City, Calif.).

[0220] PCR probes were designed by PrimerExpress software (PE Biosystems) based on the human SARP3 sequence.

[0221] To standardize the results between the different tissues, two probes, distinguished by different fluorescent labels, were added to each sample. The differential labeling of the probe for the SARP3 and the probe for 18S RNA (as an internal control) thus enabled their simultaneous measurement in the same well. Forward and reverse primers and the probes for both 18S RNA and human or murine SARP3 were added to the TaqMan Universal PCR Master Mix (PE Applied Biosystems). Although the final concentration of primer and probe could vary, each was internally consistent

within a given experiment. A typical experiment contained 200 nM each of the forward and reverse primers and 100 nM of the probe for the 18S RNA, as well as 600 nM of each of the forward and reverse primers and 200 nM of the probe for SARP3. TaqMan matrix experiments were carried out using an ABI PRISM 770 Sequence Detection System (PE Applied Biosystems). The thermal cycler conditions were as follows: hold for 2 minutes at 50° C. and 10 minutes at 95° C., followed by two-step PCR for 40 cycles of 95° C. for 15 seconds, followed by 60° C. for 1 minute.

[0222] The following method was used to quantitatively calculate SARP3 gene expression in the tissue samples, relative to the 18S RNA expression in the same tissue. The threshold values at which the PCR amplification started were determined using the manufacturer's software. PCR cycle number at threshold value was designated as CT. Relative expression was calculated as $2^{-((CTtest-CT}18S)}$ tissue of interest-(CTtest-CT18S) lowest expressing tissue in panel). Samples were run in duplicate and the averages of 2 relative expression levels that were linear to the amount of template cDNA with a slope similar to the slope for the internal control 18S were used.

[0223] The Taqman results revealed expression of SARP3 in adipose tissue from both abdomen and buttocks. Among the five individuals subjected to the high calorie, fat-rich diet, two individuals showed a significantly increased expression of SARP3 in adipose tissue from the buttocks after the eight-week diet period.

EXAMPLE 2

SARP3 Gene Expression in Mouse Tissues

[0224] Expression of mouse SARP3 mRNA was examined by Northern blot analysis in a wide panel of mouse tissues. The results indicated that expression was limited to white adipose tissue and hypothalamus.

[0225] SARP3 expression in hypothalamus was examined further by in situ staining. The results showed a diffuse pattern of expression in a subset of neurons throughout the hypothalamus.

[0226] Expression of SARP3 in mouse hypothalamus was further examined to determine whether expression was modulated by leptin treatment. Forty mice were divided into two groups maintained for five months on either a regular diet or a high fat diet, followed by administration of 5 μg leptin or phosphate buffered saline by ICV. Three hours or twenty-four hours later, the hypothalamus was removed for Northern blot analysis of SARP3 expression. The results showed a higher level of SARP3 expression in the leptin and PBS treated groups sacrificed twenty-four hours after leptin treatment as compared to the corresponding groups sacrificed three hours after leptin treatment. No significant difference was seen between the leptin-treated mice fed a regular diet or a high fat diet at either time point. However, PBS treated mice from the groups on a high fat diet showed slightly higher levels of SARP3.

[0227] Genetic mouse models of obesity (ob/ob, db/db, A^y, and tub/tub mice) were also examined for SARP3 expression in the hypothalamus. The results indicated no difference in the level of SARP3 expressed between the Ay and tub/tub mice and their respective corresponding wild type strains. In contrast, the ob/ob and db/db mice showed a lower level of SARP3 expression as compared to their respective corresponding wild type strain, which is likely due to the absence of the leptin signaling pathway in these mice.

[0228] Equivalents

[0229] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

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g atg cgg gcg gcg gcg gcg gcg ggg ggc gtg cgg acg gcc gcg ctg gcg    229
  Met Arg Ala Ala Ala Ala Ala Gly Gly Val Arg Thr Ala Ala Leu Ala
   1               5                  10                  15

ctg ctg ctg ggg gcg ctg cac tgg gcg ccg gcg cgc tgc gag gag tac      277
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Asp Tyr Tyr Gly Trp Gln Ala Glu Pro Leu His Gly Arg Ser Tyr Ser
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aag ccg ccg cag tgc ctt gac atc cct gcc gac ctg ccg ctc tgc cac      373
Lys Pro Pro Gln Cys Leu Asp Ile Pro Ala Asp Leu Pro Leu Cys His
     50                  55                  60

acg gtg ggc tac aag cgc atg cgg ctg ccc aac ctg ctg gag cac gag      421
Thr Val Gly Tyr Lys Arg Met Arg Leu Pro Asn Leu Leu Glu His Glu
 65                  70                  75                  80

agc ctg gcc gaa gtg aag cag cag gcg agc agc tgg ctg ccg ctg ctg      469
Ser Leu Ala Glu Val Lys Gln Gln Ala Ser Ser Trp Leu Pro Leu Leu
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gcc aag cgc tgc cac tcg gat acg cag gtc ttc ctg tgc tcg ctc ttt      517
Ala Lys Arg Cys His Ser Asp Thr Gln Val Phe Leu Cys Ser Leu Phe
            100                 105                 110

gcg ccc gtc tgt ctc gac cgg ccc atc tac ccg tgc cgc tcg ctg tgc      565
Ala Pro Val Cys Leu Asp Arg Pro Ile Tyr Pro Cys Arg Ser Leu Cys
        115                 120                 125

gag gcc gtg cgc gcc ggc tgc gcg ccg ctc atg gag gcc tac ggc ttc      613
Glu Ala Val Arg Ala Gly Cys Ala Pro Leu Met Glu Ala Tyr Gly Phe
    130                 135                 140

ccc tgg cct gag atg ctg cac tgc cac aag ttc ccc ctg gac aac gac      661
Pro Trp Pro Glu Met Leu His Cys His Lys Phe Pro Leu Asp Asn Asp
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ctc tgc atc gcc gtg cag ttc ggg cac ctg ccc gcc acc gcg cct cca      709
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Val Thr Lys Ile Cys Ala Gln Cys Glu Met Glu His Ser Ala Asp Gly
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ctc atg gag cag atg tgc tcc agt gac ttt gtg gtc aaa atg cgc atc      805
Leu Met Glu Gln Met Cys Ser Ser Asp Phe Val Val Lys Met Arg Ile
        195                 200                 205

aag gag atc aag ata gag aat ggg gac cgg aag ctg att gga gcc cag      853
Lys Glu Ile Lys Ile Glu Asn Gly Asp Arg Lys Leu Ile Gly Ala Gln
    210                 215                 220

aaa aag aag aag ctg ctc aag ccg ggc ccc ctg aag cgc aag gac acc      901
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225                 230                 235                 240

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Lys Arg Leu Val Leu His Met Lys Asn Gly Ala Gly Cys Pro Cys Pro
                245                 250                 255

cag ctg gac agc ctg gcg ggc agc ttc ctg gtc atg ggc cgc aaa gtg      997
Gln Leu Asp Ser Leu Ala Gly Ser Phe Leu Val Met Gly Arg Lys Val
            260                 265                 270

gat gga cag ctg ctg ctc atg gcc gtc tac cgc tgg gac aag aag aat     1045
Asp Gly Gln Leu Leu Leu Met Ala Val Tyr Arg Trp Asp Lys Lys Asn
        275                 280                 285

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ctc tac tac cct ttc ttc tac ggg gcg gca gag ccc cac tga             1135
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Lys Pro Pro Gln Cys Leu Asp Ile Pro Ala Asp Leu Pro Leu Cys His
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Thr Val Gly Tyr Lys Arg Met Arg Leu Pro Asn Leu Leu Glu His Glu
65 70 75 80

Ser Leu Ala Glu Val Lys Gln Gln Ala Ser Ser Trp Leu Pro Leu Leu
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Ala Lys Arg Cys His Ser Asp Thr Gln Val Phe Leu Cys Ser Leu Phe
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Ala Pro Val Cys Leu Asp Arg Pro Ile Tyr Pro Cys Arg Ser Leu Cys
115 120 125

Glu Ala Val Arg Ala Gly Cys Ala Pro Leu Met Glu Ala Tyr Gly Phe
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Pro Trp Pro Glu Met Leu His Cys His Lys Phe Pro Leu Asp Asn Asp
145 150 155 160

Leu Cys Ile Ala Val Gln Phe Gly His Leu Pro Ala Thr Ala Pro Pro
165 170 175

Val Thr Lys Ile Cys Ala Gln Cys Glu Met Glu His Ser Ala Asp Gly
180 185 190

Leu Met Glu Gln Met Cys Ser Ser Asp Phe Val Val Lys Met Arg Ile
195 200 205

Lys Glu Ile Lys Ile Glu Asn Gly Asp Arg Lys Leu Ile Gly Ala Gln
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Gln Leu Asp Ser Leu Ala Gly Ser Phe Leu Val Met Gly Arg Lys Val
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Asp Gly Gln Leu Leu Leu Met Ala Val Tyr Arg Trp Asp Lys Lys Asn
        275                 280                 285

Lys Glu Met Lys Phe Ala Val Lys Phe Met Phe Ser Tyr Pro Cys Ser
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ctctgcatcg ccgtgcagtt cgggcacctg cccgccaccg cgcctccagt gaccaagatc    540

tgcgcccagt gtgagatgga gcacagtgct gacggcctca tggagcagat gtgctccagt    600

gactttgtgg tcaaaatgcg catcaaggag atcaagatag agaatgggga ccggaagctg    660

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gtctaccgct gggacaagaa gaataaggag atgaagtttg cagtcaaatt catgttctcc    900

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cgcacgccgg gcagcggggc gccgagcacc gagccgtcgg ggcaggccgc aacatccagc    240

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ggt tgg cag gcc gag ccg ctg cac ggc cgc tcc tac tcc aag cca ccg      385
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Gln Cys Leu Asp Ile Pro Ala Asp Leu Pro Leu Cys His Thr Val Gly
    50                  55                  60

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Tyr Lys Arg Met Arg Leu Pro Asn Leu Leu Glu His Glu Ser Leu Ala
65                  70                  75                  80

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tgc ctg gac cga ccc atc tac ccc tgc cgc tcg ctg tgc gaa gct gcg      625
Cys Leu Asp Arg Pro Ile Tyr Pro Cys Arg Ser Leu Cys Glu Ala Ala
        115                 120                 125

cgc gcc ggc tgc gct ccg ctc atg gag gcc tac ggt ttc cct tgg ccc      673
Arg Ala Gly Cys Ala Pro Leu Met Glu Ala Tyr Gly Phe Pro Trp Pro
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gag atg ctg cac tgc cac aag ttc ccc ctg gac aac gac ctc tgc atc      721
Glu Met Leu His Cys His Lys Phe Pro Leu Asp Asn Asp Leu Cys Ile
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            180                 185                 190

cag atg tgc tcc agt gac ttt gtg gtc aag atg cgc att aag gag atc      865
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    210                 215                 220

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225                 230                 235                 240

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ctg ctg ctc acg gcc gtc tac cgc tgg gac aag aag aat aag gag atg     1105
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Gln Cys Leu Asp Ile Pro Ala Asp Leu Pro Leu Cys His Thr Val Gly
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Tyr Lys Arg Met Arg Leu Pro Asn Leu Leu Glu His Glu Ser Leu Ala
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Glu Val Lys Gln Gln Ala Ser Ser Trp Leu Pro Leu Leu Ala Lys Arg
85 90 95

Cys His Ser Asp Thr Gln Val Phe Leu Cys Ser Leu Phe Ala Pro Val
100 105 110

Cys Leu Asp Arg Pro Ile Tyr Pro Cys Arg Ser Leu Cys Glu Ala Ala
115 120 125

Arg Ala Gly Cys Ala Pro Leu Met Glu Ala Tyr Gly Phe Pro Trp Pro
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Glu Met Leu His Cys His Lys Phe Pro Leu Asp Asn Asp Leu Cys Ile
145 150 155 160

Ala Val Gln Phe Gly His Leu Pro Ala Thr Ala Pro Pro Val Thr Lys
165 170 175

Ile Cys Ala Gln Cys Glu Met Glu His Ser Ala Asp Gly Leu Met Glu
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Gln Met Cys Ser Ser Asp Phe Val Val Lys Met Arg Ile Lys Glu Ile
195 200 205

Lys Ile Asp Asn Gly Asp Arg Lys Leu Ile Gly Ala Gln Lys Lys Lys
210 215 220

Lys Leu Leu Lys Ala Gly Pro Leu Lys Arg Lys Asp Thr Lys Lys Leu
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Val Leu His Met Lys Asn Gly Ala Ser Cys Pro Cys Pro Gln Leu Asp
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Asn Leu Thr Gly Ser Phe Leu Val Met Gly Arg Lys Val Glu Gly Gln
260 265 270

Leu Leu Leu Thr Ala Val Tyr Arg Trp Asp Lys Lys Asn Lys Glu Met
275 280 285

Lys Phe Ala Val Lys Phe Met Phe Ser Tyr Pro Cys Ser Leu Tyr Tyr
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Pro Phe Phe Tyr Gly Ala Ala Glu Pro His
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<211> LENGTH: 945
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

-continued

<400> SEQUENCE: 6

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ggccgctcct actccaagcc accgcagtgc ctcgacatcc ccgccgatct gccgctctgt 180

cacacggtgg gctacaagcg catgcggctg cccaacctgc tggagcacga gagcctggcc 240

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accaggtct tcctctgctc gctcttcgct ccogtctgcc tggaccgacc catctacccc 360

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tccctctact accctttttt ctatggggca gctgaacccc actga 945

What is claimed is:

1. A method for identifying a compound capable of treating a metabolic disorder, comprising assaying the ability of the compound to modulate an SARP3 nucleic acid expression or SARP3 polypeptide activity, thereby identifying a compound capable of treating a metabolic disorder.

2. The method of claim 1, wherein the metabolic disorder is selected from the group consisting of obesity, overweight, diabetes, insulin resistance, cachexia, and anorexia.

3. The method of claim 1, wherein the ability of the compound to modulate a SARP3 nucleic acid expression or a SARP3 polypeptide activity is determined by detecting a SARP3 activity of a cell.

4. The method of claim 1, wherein the SARP3 is selected from the group consisting of:

a) a polypeptide comprising an amino acid sequence which is at least 90 percent identical to the amino acid sequence of SEQ ID NO:2 or 5; and

b) a naturally occurring allelic variant of a polypeptide consisting of the amino acid sequence of SEQ ID NO:2, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a complement of a nucleic acid molecule consisting of SEQ ID NO: 1 in 6×SSC at 45° C., followed by one or more washes in 0.2×SSC, 0.1% SDS at 65° C.

5. A method for identifying a compound capable of modulating a SARP3 mediated metabolic activity, comprising:

(a) contacting a cell which expresses SARP3 with a test compound; and

(b) assaying the ability of the test compound to modulate the expression of a SARP3 nucleic acid or the activity of a SARP3 polypeptide, thereby identifying a compound capable of modulating a SARP3 mediated metabolic activity.

6. A method for identifying a compound capable of modulating a SARP3 mediated metabolic activity, comprising:

(a) contacting a composition comprising a polypeptide comprising the amino acid sequence of SEQ ID NO:2 or 5 with a test compound; and

(b) assaying the ability of the test compound to modulate the activity of the polypeptide, thereby identifying a compound capable of modulating a SARP3 mediated metabolic activity.

7. The method of claim 5, wherein the SARP3 is a polypeptide selected from the group consisting of:

a) a polypeptide comprising an amino acid sequence which is at least 90 percent identical to the amino acid sequence of SEQ ID NO:2 or 5; and

b) a naturally occurring allelic variant of a polypeptide consisting of the amino acid sequence of SEQ ID NO:2, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a complement of a nucleic acid molecule consisting of SEQ ID NO: 1 in 6×SSC at 45° C., followed by one or more washes in 0.2×SSC, 0.1% SDS at 65° C.

8. The method of claim 6, wherein the SARP3 is a polypeptide selected from the group consisting of:

a) a polypeptide comprising an amino acid sequence which is at least 90 percent identical to the amino acid

sequence of SEQ ID NO:2 or 5, wherein said percent identity is calculated using the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4; and

b) a naturally occurring allelic variant of a polypeptide consisting of the amino acid sequence of SEQ ID NO:2, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a complement of a nucleic acid molecule consisting of SEQ ID NO: 1 in 6×SSC at 45° C., followed by one or more washes in 0.2×SSC, 0.1% SDS at 65° C.

9. A method for modulating a SARP3 mediated metabolic activity comprising contacting a cell or a tissue expressing the SARP3 with a SARP3 modulator, thereby modulating the SARP3 mediated metabolic activity.

10. The method of claim 9, wherein the compound or modulator is selected from the group consisting of a small molecule SARP3 agonist, a small molecule SARP3 antagonist, a small molecule SARP3 inverse agonist, an anti-SARP3 antibody, an antisense SARP3 molecule, and a SARP3 ribozyme.

11. The method of claim 9, wherein the SARP3 mediated metabolic activity comprises an activity selected from the group consisting of:

a) the ability to modulate lipid metabolism;

b) the ability to modulate glucose metabolism;

c) the ability to modulate insulin metabolism;

d) the ability to modulate adipocyte growth;

e) the ability to modulate the differentiation of adipose cell progenitors into adipocytes; and

f) the ability to modulate programmed cell death.

12. The method of claim 1, wherein the ability of the compound to modulate an SARP3 nucleic acid expression or SARP3 polypeptide activity is determined by detecting modulation of any one of:

a) the level of β-catenin;

b) leptin or insulin sensitivity;

c) food intake, body weight change, or glucose tolerance;

d) hyperplastic growth;

e) cell differentiation;

f) programmed cell death; and

g) hypertrophic growth.

13. The method of claim 5, wherein the ability of the compound to modulate an SARP3 nucleic acid expression or SARP3 polypeptide activity is determined by detecting any one of:

a) the level of β-catenin;

b) leptin or insulin sensitivity;

c) food intake, body weight change, or glucose tolerance;

d) hyperplastic growth;

e) cell differentiation;

f) programmed cell death; and

g) hypertrophic growth.

14. The method of claim 6, wherein the ability of the compound to modulate an SARP3 nucleic acid expression or SARP3 polypeptide activity is determined by detecting any one of:

a) the level of β-catenin;

b) leptin or insulin sensitivity;

c) food intake, body weight change, or glucose tolerance;

d) hyperplastic growth;

e) cell differentiation;

f) programmed cell death; and

g) hypertrophic growth.

15. A method for treating a subject having a metabolic disorder characterized by aberrant SARP3 polypeptide activity or aberrant SARP3 nucleic acid expression, comprising administering to the subject a SARP3 modulator, thereby treating the subject having a metabolic disorder.

16. The method of claim 15, wherein said metabolic disorder is selected from the group consisting of obesity, overweight, diabetes, insulin resistance, cachexia, and anorexia.

17. The method of claim 15, wherein the modulator is selected from the group consisting of a small molecule SARP3 agonist, a small molecule SARP3 antagonist, a small molecule SARP3 inverse agonist, an anti-SARP3 antibody, an antisense SARP3 molecule, and a SARP3 ribozyme.

18. A pharmaceutical formulation for the treatment of metabolic disorders, comprising a compound selected from:

a) a compound that activates SARP3 polypeptide activity or SARP3 nucleic acid expression, and

b) a compound that inhibits SARP3 polypeptide activity or SARP3 nucleic acid expression;

wherein the formulation further comprises a pharmaceutically acceptable carrier.

19. The pharmaceutical formulation of claim 18, wherein the compound is selected from the group consisting of a small molecule SARP3 agonist, a small molecule SARP3 antagonist, a small molecule SARP3 inverse agonist, an anti-SARP3 antibody, an antisense SARP3 molecule, and a SARP3 ribozyme.

20. The pharmaceutical formulation of claim 19 in which the compound is an oligonucleotide encoding an antisense or ribozyme molecule that targets SARP3 transcripts and inhibits translation or an oligonucleotide that forms a triple helix with the promoter of the SARP3 gene and inhibits transcription.

* * * * *

EXHIBIT B-2

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/338,604	01/08/2003	Haiyan Xu	MP101-250P1RM	2607

32116 7590 05/16/2006

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SUITE 3800
CHICAGO, IL 60661

EXAMINER	
CHANDRA, GYAN	

ART UNIT	PAPER NUMBER
1646	

DATE MAILED: 05/16/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Applicants: Kiran K. Chada et al.
Serial No.: 10/768,566
Filed: January 29, 2004
Exhibit B-2

Office Action Summary	Application No.	Applicant(s)	
	10/338,604	XU, HAIYAN	
	Examiner	Art Unit	
	Gyan Chandra	1648	

-- *The MAILING DATE of this communication appears on the cover sheet with the correspondence address* --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *01 March 2006*.

2a)☐ This action is FINAL. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-20* is/are pending in the application.

4a) Of the above claim(s) *1-8 and 12-20* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *9-11* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *4/15/2005*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

Applicant's election with traverse of Group II, claims 9-11 and species "the ability to modulate lipid metabolism" in the reply filed on 03/01/2006 is acknowledged. However, Applicant did not distinctly and specifically points out the supposed error in the restriction requirement.

The requirement is still deemed proper and is therefore made FINAL.

Status of Application, Amendments, And/Or Claims

Claims 1-20 are pending. Claims 1-8, and 12-20 are withdrawn from further consideration as being drawn to a nonelected invention.

Claims 9-11 are examined on the merit to the extent that they read on the elected species the ability to modulate lipid metabolism.

Information Disclosure Statement

The information disclosure statement (IDS) filed on 4/15/2005 has been considered.

Specification

The disclosure is objected to because it contains an embedded hyperlink and/or other form of browser-executable code, see page 59, lines 8 and 11. Applicant is required to delete the embedded hyperlink and/or other form of browser-executable code. See MPEP § 608.01.

Claim Objections

Claim 9 is objected for the use of many abbreviated phrases (SARP3), which should be described for the first time followed by an abbreviated form placed in a bracket. Appropriate correction is required.

Claim Rejections - 35 USC § 101 and 35 USC § 112

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 9-11 are rejected under 35 U.S.C. § 101 because the claimed invention is not supported by either a credible, specific and substantial asserted utility or a well established utility. Novel biological molecules lack a well established utility and must undergo extensive experimentation.

Specifically, claims 9-11 are directed to a method of modulating a SARP3 mediated activity comprising contacting a cell or a tissue that expresses SARP3 with a SARP3 modulator that can modulate lipid metabolism. However, the instant specification does not teach any significance or functional characteristics of the polypeptide SARP3. The specification also does not disclose any specific methods or

working examples for modulating lipid metabolism. The specification discloses in general how a skilled artisan can different screening methods to identify a compound for any known protein that has a known biological function, but fails to show if the instantly claimed polypeptide has any such an impact. Since the utility is not presented in mature form and significant further research is required, the utility is not substantial. The specification asserts the following as patentable utilities for the claimed polypeptide SARP3:

1) to prepare an antibody (pg 63-66);

2) to screen modulators of SARP3 activity (pg 8-20);

3) to detect metabolic disorders (page 20-28); and

4) to treat subjects suffering from metabolic disorders (pg 34-41)

Each of these shall be addressed in turn.

1) to prepare an antibody. This asserted utility is not specific or substantial. An antibody can be prepared for any protein and it is a routine in the art. Further, the specification discloses nothing specific or substantial for the SARP3 polypeptide where this antibody can be used. The use of an antibody for binding the protein against which it is raised is of the type of experimentation that does not meet the requirements of 35 USC § 101.

2) to screen modulators of SARP3. This asserted utility is not specific or substantial. The specification discloses methods to screen for a compound that can modulate a polypeptide SARP3 activity. Since the polypeptide itself does not have any

known activity, the methods of screening using the SARP3 are not presented in a ready to use, real-world application, and the asserted utility is not substantial.

3) *to detect metabolic disorders.* These asserted utilities are not substantial. The disclosed utility is not substantial because the specification provides no information that the polypeptide SARP3 can accomplish this. The specification teaches how one can use a marker to detect its presence in a sample. However, in the absence of any biological relevance or disease association, mere presence of the SARP3 polypeptide or mRNA encoding a SARP3 polypeptide does not provide a specific and substantial utility for detecting a metabolic disorder. Significant further research would be required of the skilled artisan to perform experiments to establish, if the protein or encoding nucleic acid could be used for detecting a metabolic disorder. Since the asserted utility is not presented in a ready to use, real-world application, the asserted utility is not substantial.

4) *to treat subjects suffering from metabolic disorders.* Since the polypeptide SARP3 does not have any disclosed biological function and it is expressed in adipose tissue in a mouse model (Example 2, page 68), this does not establish its biological role for any therapeutic intervention. The disclosed polypeptide is an orphan protein for which a real world biological function has yet to be identified. Therefore, treatment of a metabolic disorder using a SARP3 modulator does not have a substantial support. Also, Chang et al. (IDS, Human Mol. Gen 8: 575-583, 1999) disclose that SFRP5, also know as SARP3, is expressed in retinal pigment epithelium (RPE) and may have some role in

wnt signaling. Therefore, any biological relevance of the polypeptide is far from a well established use.

Therefore, the asserted utility of the instantly claimed invention is not established as a substantial and real-world use. Thus, the proposed use of the claimed method is simply a starting point for further research and investigation into potential uses of the polypeptide and any compound that would modulate its activity. See Brenner v. Manon, 148 U.S.P.Q. 689 (Sup. Ct, 1966), wherein the court held that.

Claims 9-11 are also rejected under 35 U.S.C. 112, first paragraph. Specifically, since the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility for the reasons set forth above, one skilled in the art clearly would not know how to use the claimed invention. Even if the specification disclosed any utility for the claimed polypeptide, it would not enable for a method of modulating a SARP3 mediated lipid metabolism.

The first paragraph of 35 U.S.C. 112 states, "The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same...". The courts have interpreted this to mean that the specification must enable one skilled in the art to make and use the invention without undue experimentation. The courts have further interpreted undue experimentation as requiring "ingenuity beyond that to be expected of one of ordinary skill in the art" (Fields v. Conover, 170 USPQ 276

(CCPA 1971)) or requiring an extended period of experimentation in the absence of sufficient direction or guidance (In re Colianni, 195 USPQ 150 (CCPA 1977)). Additionally, the courts have determined that "... where a statement is, on its face, contrary to generally accepted scientific principles", a rejection for failure to teach how to make and/or use is proper (In re Marzocchi, 169 USPQ 367 (CCPA 1971). Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 U.S.C. 112, first paragraph, have been described in In re Colianni, 195 USPQ 150, 153 (CCPA 1977) and have been clarified by the Board of Patent Appeals and Interferences in Ex parte Forman, 230 USPQ 546 (BPAI 1986).

Among the factors are the nature of the invention, the state of the prior art, the predictability or lack thereof in the art, the amount of direction or guidance present, the presence or absence of working examples, the breadth of the claims, and the quantity of experimentation needed. The instant disclosure fails to meet the enablement requirement for the following reasons:

The Nature of the Invention: The claims are drawn to a method of modulating a SARP3 mediated activity comprising contacting a cell or a tissue that expresses SARP3 with a SARP3 modulator that can modulate lipid metabolism.

The state of the prior art and the predictability or lack thereof in the art:

Lipids and fatty acids play major role in energy balance, hormone synthesis and many metabolic activities. Ranneries et al. (Am. J. Physiology 274: E155- E161, 1998) suggest that any imbalance in fatty acid metabolism can lead to many diseases and disorders. They state that obesity develops due to an interaction between genetic

components and certain environmental factors such as a high fat diet (page E155, 1st paragraph of the left column). It is well known in the art that the low density lipid and triglycerides are high risk factors for many cardio-vascular diseases. Further, obesity is a risk factor for diabetes which is a polygenic disease. Chang et al disclose that the SFRP family comprise many proteins such as SFRP 1-5 (Table 1 on page 576) based on their structure homology. There is no suggestion if any of these proteins can modulate lipid metabolism. Rather Chang et al indicate a possible role of the claimed polypeptide in wnt signaling in eye retina. Therefore, the art indicates that SARP3 is not involved in lipid metabolism.

The amount of direction or guidance present and the presence or absence of working examples: Given the teachings of unpredictability found in the art, detailed teachings are required to be present in the disclosure in order to enable the skilled artisan to practice the claimed invention. These teachings are absent. There is no discussion of how SARP3 can play a role in modulating lipid metabolism and thus, the specification fails to support the assertion of the therapeutic activities of the protein. One of skill in the art would have no starting point to determine how to modulate lipid metabolism. While the specification contains a general discussion on how to screen a compound that could bind or interact with a protein having a known biological function, the specification is totally devoid of any working example in which SARP3 is demonstrated to be involved in lipid metabolism so that it can be applied for treating diabetes or metabolic disorders in a diabetic subject for its contemplated use. The prior

art does not suggest or indicate that the instantly claimed polypeptide SARP3/SFRP5 has any role modulating lipid activity in a subject.

The breadth of the claims and the quantity of experimentation needed: Because the claims encompass a method of modulating a SARP3 mediated activity comprising contacting a cell or a tissue that expresses SARP3 with a SARP3 modulator that can modulate lipid metabolism, in the light of the teachings of the unpredictability found in the art discussed and because of the supra lack of sufficient teachings in applicants disclosure to overcome those teachings, it would require undue experimentation by one of skill in the art to be able to practice the claimed invention.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Gyan Chandra whose telephone number is (571) 272-2922. The examiner can normally be reached on 9:00-5:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Nickol can be reached on (571) 272-0835. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Gyan Chandra, Ph.D.
Art Unit 1646
8 May 2006
Fax: 571-273-2922

Eileen B. O'Hara

EILEEN B. O'HARA
PRIMARY EXAMINER

Notice of References Cited	Application/Control No. 10/338,604	Applicant(s)/Patent Under Reexamination XU, HAIYAN	
	Examiner Gyan Chandra	Art Unit 1646	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-			
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Ranneries et al., Am. J. Physiology 274: E155- E161, 1998.
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

EXHIBIT B-3

Statement of where items B1 and B2 were entered into the record by the Examiner

Xu et al., U.S. Patent Application Publication No. 2003/0143610 A1, published July 31, 2003 (B1) was entered into the record by the Examiner in the Office Action issued April 19, 2006 in connection with the subject application.,

Office Action issued May 16, 2006 in connection with U.S. Serial No. 10/338,604 (B2) was acknowledged by the Examiner in the Advisory Action issued March 5, 2007 in connection with the above-identified application.

EXHIBIT C

69014-B

G

UNITED STATES PATENT AND TRADEMARK OFFICE

UNDER SECRETARY OF COMMERCE FOR INTELLECTUAL PROPERTY AND
DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE

JANUARY 27, 2005

PTAS

102802699A

COOPER & DUNHAM LLP
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NEW YORK, NY 10036

FEB 1

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THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT DIVISION OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE ASSIGNMENT SEARCH ROOM ON THE REEL AND FRAME NUMBER REFERENCED BELOW.

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RECORDATION DATE: 07/28/2004

REEL/FRAME: 015613/0506
NUMBER OF PAGES: 4

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).
DOCKET NUMBER: 69014-B

ASSIGNOR:
CHADA, KIRAN K.

DOC DATE: 07/03/2003

ASSIGNOR:
CHOUINARD, ROLAND

DOC DATE: 07/03/2003

ASSIGNOR:
ASHAR, HENA

DOC DATE: 07/03/2003

ASSIGNOR:
SAYED, ABU

DOC DATE: 07/07/2004

ASSIGNEE:
HMGENE INC.
675 HOES LANE RESEARCH TOWER
ROOM R-603
PISCATAWAY, NEW JERSEY 08854

Applicants: Kiran K. Chada et al.
Serial No.: 10/768,566
Filed: January 29, 2004
Exhibit C

SERIAL NUMBER: 10768566 FILING DATE: 01/29/2004
PATENT NUMBER: ISSUE DATE:
TITLE: METHOD OF TREATING OBESITY AND METABOLIC DISORDERS RELATED TO EXCESS ADIPOSE TISSUE BY ADMINISTRATION OF SFRP-5 PEPTIDE

PAULA MCCRAY, EXAMINER
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

07-30-2004

Docket No. 69014-B

FORM PTO-1595 (Substitute) 7/28/04 RECORD P

(Rev. 8-96)

102802699

U.S. DEPARTMENT OF COMMERCE

Patent and Trademark Office

To the Honorable Commissioner of Patents and Trademarks: Please ... or copy thereof.

1. Name of conveying party(ies):
Kiran K. Chada,
Roland Chouinard,
Hena Ashar and
Abu Sayed

Additional name(s) of conveying party(ies) attached? ☐ Yes ☒ No

2. Name and address of receiving party(ies):

Name: HMGene Inc.

Internal Address:

Street Address: 675 Hoes Lane Research Tower
Room R-603

City/State/Zip: Piscataway, NJ 08854

Additional name(s) & address(es) attached? ☐ Yes ☒ No

3. Nature of Conveyance:

☒ Assignment ☐ Merger
☐ Security Assignment ☐ Change of Name
☐ Other

Execution Date(s): July 3, 2003; July 3, 2003; July 3, 2003; July 7, 2004

4. Application number(s) or patent number(s): If this document is being filed together with a new application, the execution date(s) of the application is (are):

A. Patent Application No.(s) U.S. Serial No. 10/768,566, filed January 29, 2004

B. Patent No.(s)

Additional numbers attached? ☐ Yes ☒ No

2004 JUL 23 AM 7:23
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5. Name and address of party to whom correspondence concerning document should be mailed:

Name: Gary J. Gershik

Internal Address:

Street Address: Cooper & Dunham LLP
1185 Avenue of the Americas

City/State/Zip: New York, New York 10036

6. Total number of applications and patents involved: 1

7. Total fee (37 CFR §3.41):.....$ 40.00

☐ Enclosed

☒ Authorized to be charged to deposit account

8. Deposit account number:

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9. Statement and signature.

To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document.

Gary J. Gershik — Name of Person Signing

Gary J. Gershik — Signature Reg. No. 39,992

July 23, 2004 — Date

I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to:
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Assignment

In consideration of One Dollar ($1.00), and other good and valuable considerations, the receipt of which is hereby acknowledged, we, the undersigned,

Kiran K. Chada residing at 116 Pinehurst Avenue, Apt. G22, New York, NY 10033;
Roland Chouinard residing at 164-B Pleasantview Drive, Piscataway, NJ 08854;
Hena Ashar residing at 239 W. Prescott, Edison, NJ 08820 and
Md. Abu Sayed residing at 1669 Spring Park Walk, Cincinnati, OH 45215

Hereby sell, assign and transfer to HMGene, Inc. a corporation of the State of Delaware having a place of business at 675 Hoes Lane Research Tower, Room R-603 NJ 08854 in the County of and State of New Jersey its successors, assigns and legal representatives, the entire right, title and interest for all countries, in and to any and all inventions which are disclosed and claimed, and any and all inventions which are disclosed but not claimed, in the application for United States Patent, which has been executed by the undersigned on July 3, 2003; July 3, 2003; July 3, 2003; July 7, 2004 and is entitled

METHODS OF TREATING OBESITY AND METABOLIC DISORDERS RELATED TO EXCESS ADIPOSE TISSUE BY ADMINISTRATON OF s-FRP-5 PEPTIDE
(U.S. Serial No. 10/768,566 , filed January 29, 2004, continuation-in-part of U.S. Serial No. 10/630,423, filed July 29, 2003, claiming benefit of U.S. Provisional Application No. 60/478,206, filed June 12, 2003 and U.S. Provisional Application No. 60/398,785, filed July 29, 2002)

and in and to said application and all divisional, continuing, substitute, renewal, reissue, and all other applications for U.S. Letters Patent or other related property rights in any and all foreign countries which have been or shall be filed on any of said inventions disclosed in said application; and in and to all original and reissued patents or related foreign documents which have been or shall be issued on said inventions;

Authorize and request the Commissioner of Patents of the United States to issue to said Assignee, the corporation above named, its successors, assigns and legal representatives, in accordance with this assignment, any and all United States Letters Patent on said inventions or any of them disclosed in said application;

Agree that said Assignee may apply for and receive foreign Letters Patent or rights of any other kind for said inventions, or any of them; and may claim, in applications for said foreign Letters Patent or other rights, the priority of the aforesaid United States patent application under the provisions of the International Convention of 1883 and later modifications thereof, under the Patent Cooperation Treaty, under the European Patent Convention or under any other available international agreement; and that, when requested, without charge to, but at the expense of, said Assignee, its successors, assigns and legal representatives, to carry out in good faith the intent and purpose of this assignment, the undersigned or the undersigned's executors or administrators will, for the United States and all foreign countries, execute all divisional, continuing, substitute, renewal, reissue, and all other patent applications or other documents on any and all said inventions; execute all rightful oaths, assignments, powers of attorney and other papers; communicate to said Assignee, its successors, assigns and representatives, all facts known and documents available to the undersigned relating to said inventions and the history thereof; testify in all legal proceedings; and generally do everything possible which said Assignee, its successors, assigns or representatives shall consider desirable for aiding in securing, maintaining and enforcing proper patent protection for said inventions and for vesting title to said inventions and all applications for patents or related foreign rights and all patents on said inventions, in said Assignee, its successors, assigns and legal representatives; and

Covenant with said Assignee, its successors, assigns and legal representatives that no assignment, grant, mortgage, license or other agreement affecting the rights and property herein conveyed has been made to others by the undersigned, and that full right to convey the same as herein expressed is possessed by the undersigned.

Date: 7/3, 2003

Witness: [signature]

Camille Vaughn [printed name]

454 Lewis St., Somerset, NJ [address]
08873

[L.S.]

Kiran K. Chada

Date: 7/3, 2003

Witness: [signature]

Camille Vaughn [printed name]

454 Lewis St., Somerset, NJ [address]
08873

[L.S.]

Roland Chouinard

Date: 7/3 2003

Witness: Camille Vaugh [signature]

Camille Vaughn [printed name]

454 Lewis St., Somerset, NJ [address]

08873

Hena Ashar [L.S.]

Date: July 7th 2004

Witness: Jared Smith [signature]

Jared Smith [printed name]

429 McAlpin Ave. #1 [address]

Cincinnati, OH 45220

Md. Abu Sayed [L.S.]

EXHIBIT D

RELATED PROCEEDINGS APPENDIX

-NONE-